Field release of transgenic crops in Switzerland

an ecological risk assessment of vertical gene flow

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1. The process of domestication of crop plants

1.1. Definition of domestication

Domestication processes lead to genetical modification, so that wild plants ± gradually become cultivated plants. Modification is often important to such an extent, that at the limit, the cultivar cannot survive by itself in the wild.
Evolution is basically a process of gene substitution, gene mutation, gene recombination, often enhanced by hybridization and polyploidisation. This is the same for crop plants: the basic process is gene substitution, either at diploid or at polyploid level.

Domestication basically means modification by human selection in order to enhance handling (seed storage, cultivation, harvest procedures, product storage), yield, safety and sometimes also aesthetics of the product. Domestication has to be defined as a syndrome with numerous characters. Only a selection of those characters is achieved depending on the morphology and biology of a given cultivar.

Definitions vary according to the authors views about the process of domestication:
Domestication is usually described as "changes in adaptation that insure total fitness in habitats especially prepared by man for his cultigen" (De Wet 1981 in Raamsdonk van 1993). Small (1984) characterised domestication as a process of co-evolution of cultivated plants and man. He included in this process evolutionary forces such as mutation, selection, genetic drift and hybridization. Hammer (1984) included in addition some syndrome characters as loss of natural dispersal mechanisms, larger propagules, loss of mechanical means of protection, loss of toxic or repellent chemicals, colour changes in fruit or seeds, homogenised and rapid germination and simultaneous ripening. A much larger list of breeding objectives is given by Simmonds (1979).

Domestication syndrome characters can be summarised as follows:
- Enhanced yield by enlargement of edible parts such as leaves, fruits, seeds, roots etc.
- Loss of natural distribution mechanisms for the diaspores
- Heavier or bigger diaspores
- Loss of defence devices (mechanical and chemical) against predators
- Simultaneous and accelerated germination

1.2. Origin of domestication

Sources of evidence about the origin of domestication are still accumulating. Major trends show clearly that in the Old World domestication started with early Neolithic farming villages that developed in the Near East by 7600 - 7000 BC. Early domestication in this „Nuclear Area“ is based on a relatively small number of local grain plants.

Evidence for domestication of einkorn wheat and emmer wheat, flax, bean or lentil (9200-7500 BC) begins in the Near East at least 8000 to 9000 years ago. In the New world, similar dates have been obtained for *Phaseolus* in Peru and for *Cucurbita* in Mexico. Surprisingly, the domestication of maize has been successful much later, i.e. around 5000 BC.

Rice, *sorghum*, soybean and sugarcane were probably domesticated in the last millennium BC, while others were cultivated only AC**.** On the other hand, at least three cultures are judged to be very recent. Sugar beet was developed in Europe during the 17th century, while rubber and oil palm were domesticated for the industrial world at the end of the century. The culture of forage grasses and clover is relatively new and can be regarded with numerous other cases as being still in a transition state of domestication.

Archaeological evidence suggests that wild grasses played a substantial role in the nutrition of proto-agriculture. Seeds were harvested on a large scale (*Setaria* in Mexico or wild emmer, einkorn and barley in the Near East) before the grasses were domesticated (Harlan et al. 1973). As long as human activity is confined to harvesting, the genetic effect on wild populations is negligible. This is the seeds that escape the harvester that contribute to the next generation. As soon as man starts to plant what he has harvested, the situation changes drastically. Automatic selection for better characteristics is set up (table 1).

Table 1 : Adaptation syndromes resulting from automatic selection due to planting harvested seed (Harlan et al. 1973).

Selection pressure associated with harvesting result in :
1) increase in % seed recovering
2) increase in seed production

Selection pressure associated with seedling competition result in :
1) increase in seedling vigour
2) more rapid germination

Selection pressure associated with tillage and other disturbances result in:
production of weed varieties.

The domesticated traits are generally maladjusted in nature, causing plants with such characteristics to compete ineffectively with those that retained the wild type characteristics (Newmann 1990).

Domestication was often accompanied by extinction. There is no doubt that some cultures have disappeared without leaving any trace.

As a general rule, domestication of crops (and without any doubt extinction) has not been limited to certain periods of the history. Domestication reflects continuous changes corresponding to needs for new and better agricultural products for the human society.

Important characteristics of cultivar evolution for selected Swiss crops
(from Simmonds 1988)

Family and Genus	English name	Area	Age of culture	Reproduction mode	Ploïdy level
Chenopodiaceae *Beta vulgaris*	Sugar-beet	Europe	R	O/SP	2x
Cruciferae *Brassica napus*	Rape	Europe	L	I>O/SP	AL 4x
Gramineae *Zea mays*	Maize	America	E or A?	O/SP	2x
Gramineae *Triticum aestivum*	Wheat	Middle East	A	I/SP	AL 6x
Rosaceae *Malus domestica*	Apple	East Asia	E	O/CL	2x, 3x
Vitaceae *Vitis vinifera*	Grape	West Asia	E	O/CL	2x
Solanaceae *Solanum tuberosum*	Potato	South America (Andean)	E	O/CL	2x-5x AU, 4x
Leguminosae *Medicago sativa*	Alfalfa	Middle East	E	O/SP	AU 4x

Legend of the table :

In this table (based on Simmonds 1967a), the area column indicates the centre of origin.

The age of the culture, if known, is classified as follows:
A Ancient, more than 5000 years BC
E Early , between 0-5000 BC
L Late, 0-1700 AC
R Recent, 1700 AC

The column reproduction indicates type of breeding or reproduction:
O Outbreeding population which suffers from inbreeding depression
I Fixed inbreeding line always self-fertilised or tolerating self-fertilisation
I>O In- or outbreeding population closer to inbreeding
SP Seed propagated
VP Vegetatively propagated
MP Mixed propagation by various reproduction systems, both seed dispersal or vegetative systems

The last column indicates ploïdy level and autogamy (AU) or allogamy (AL).

1.3. Where has domestication appeared ?

Cultivated plants are derived from wild species by seed selection of early farmers. They have maintained only a part of the genetic diversity by selecting those characters which made the harvesting and the farming easier. This process arrived in an area called centre of origin.

In the centre of origin of cultivated plant (Jacot and Jacot 1994):

- wild relatives coexist with the crops
- gene flux between the two compartments is controlled by genetic factors and by human activity
- plants derived from backcrosses and self-fertilisation of F1 can result in favourable crop phenotypes being selected for future use by farmers.

1.4. Who is responsible for domestication ?

Farmers and scientists are responsible for the domestication and the evolution of crops.
For many hundred years crop evolution has been in the hands of farmers, today in many parts of the world responsibility has been taken over by professional plant breeders.

1.5. Which plants are concerned ?

There are about 230 crops (including also less important crops) (Simmonds 1976). They belong to 64 families and 180 genera. Heywood et al. (1995) counted no less than 397 crop plants, cultivars and their wild relatives.

All the domesticated species are relatives of wild species but in very different ranges. We know more than 60 wild relatives for tobacco, sunflower, alfalfa, potato, cherry. We can find less than 10 species for soya, wheat, maize and sugar-beet. Maize has really only one wild relative species, teosinte, which grows in Mexico and in Guatemala where it can cross with maize in seed production fields.

Under domestication, differences between crops and wild relatives have sometimes a small genetic basis and selection can be characterised as disruptive (van Raamsdonk 1993). Genetic drift (or founder effect) is an important factor in plant domestication, since frequently small samples were propagated for sowing the next generation. This founder effect is assumed to be responsible for loss of isozyme alleles in some species (van Raamsdonk 1993).

1.6. What’s the process of domestication ?

Weeds and crops are often closely interrelated and differential characters vary.
Evolution mechanisms are the same in crops and in natural species. They include the geographic and ecological differentiation of populations regarding their reproductive adaptation by gene substitution and polyploidy. Crop breeding is often a mixture of natural and anthropogeneous processes.

In the history of plant breeding several stages can be recognised. For centuries local farmers harvested and propagated plant material for the next season, thus creating land races. Until here domestication is still non-intentional or unconscious. For many decades classical breeding has been undertaken by research institutes on all levels, in more or less close collaboration with farmers. It is only recently that seed firms started to develop modern cultivars designed for global use. Methods include interspecific crosses and back-crossing and induced mutation. The production of F1-hybrid cultivars by crossing inbreeding lines using heterosis effects are currently in the trend. Modern methods based on pollination ecology have decreased the abortion effect and levels of F1 sterility following hybridisation and have hence increased the importance of successful hybridisation.

Selection for early vernalisation and early flowering in ornamentals may occasionally lead to some kind of seasonal isolation as a by-product (van Raamsdonk, 1993).

Whereas survival of some crops is fully related to human activity, others still possess various independent survival strategies resembling those of their wild relatives (Newmann, 1990).

2. Effects on neighbouring natural area: gene transfer and cross-hybridisation

Genetic engineering is obviously different and more powerful than traditional breeding. The question is whether this difference and power translate into greater, more serious risks, or whether the risks are different from those of traditionally bred products. At this early stage in the use of the technology, it is impossible to answer this question with certainty.

Gene escape depends on many factors: whether the crop is allowed to flower; how far its pollen travels, success of fertilisation, extent of seed dispersal, seed survival and so on. Even if a gene does escape, its future may be bleak if it handicaps its new host (Young 1989).

We will concentrate here on the hybridization process and pollen dispersal.
The first question is:

Are there any differences between conventionally bred plants and transgenic plants for the potential of cross-hybridization ?

2.1. General introduction

Sexually compatible plants reproduce normally, which means that an egg in one plant can be fertilised by pollen from another plant and that the fertilised egg develops into a viable, fertile offspring. Populations may vary in their degree of compatibility. Some plants are self-compatible but some are self-incompatible as a means of enforcing out-crossing. Generally, sexually compatible plants are closely related and are usually classified in the same species. Organisms in the same genus belonging to different species may occasionally interbreed as well. Occasionally, plants not classified as closely related species may well interbreed (for example *Geum* div. spec., wheat and one of its parental partners *Aegilops*).

Sexual reproduction is initiated when pollen from one plant lands on the stigma of a flower of a sexually compatible plant. Pollen grains produce tubes that grow into the female part of the flower and from which the plant's eggs are fertilised. The fertilised eggs, containing genes from the pollen and the egg, grow into embryos within seeds. Where these seeds contain genetic contributions from two varieties of parental plants that differ in one or more traits, they are called hybrids. When regarding transgenic plant crossing with a wild species, half the hybrid's genes will come from the transgenic crop and half from the compatible wild species. If these hybrids produce viable, fertile seeds that grow into fertile plants, it may cross again with the wild/weedy relative, moving the gene into the wild/weedy population which is called introgression.

Introgression or back-crossing is also important when crops and wild relatives are interfertile and crosses may result in weedy intermediates as part of the so-called crop-weed complexes (Raamsdonk van 1993). When studying the evolution of crops, interactions between weeds and crops are obviously important factors as Pickersgill (1981) point out 3 different pathways arose in the origin of the crop plant (see chapter 3.3.).

Crops can be divided into facultative and obligate cultigens (Sukopp and Sukopp 1993). Facultative cultigens may not differ genetically from wild taxa and many such species have been transferred to new regions where they have been naturalised. Obligate cultigens are highly domesticated species that have generally lost the ability to survive in natural habitats. Most crop species that are the targets of genetic modification belong to this group. Hybridization and introgression between obligate cultigens and wild species may remove deleterious (in natural habitats) crop traits, or add traits from the wild species, which allows such crops to escape from agricultural systems. Where introgression has occurred, the hybrids tend to colonise agricultural or disturbed habitats. In addition, if a certain genetic modification of the crop alters the probability of hybrid formation or the performance of hybrids, then natural and semi-natural habitats, as well as man-made habitats may be invaded by hybrids carrying the modification.

2.1.1. Definition

"Hybridization" can be defined as the cross-breeding of genetically dissimilar individuals. These individuals may differ in a few or in numerous genes or be very different genetically. They may belong to various populations or races of the same taxonomic species (interspecific hybridization) or to different species (intergeneric hybridization). Hybridization is the starting point for two established evolutionary processes in plants : introgression and polyploidy formation.

2.1.2. Factors of hybridization

The probability of successful pollination depends on a great number of interrelated factors, such as:

- level of pollen production of (transgenic) plant
- rate of self- and cross-fertilisation of receptor plant
- rate of dispersion of donor pollen
- properties of pollinating agents
- spatial distance between pollen donor and wild recipient population
- local density of recipient population
- difference in phenology between crop and wild population

Hybridization can only occur if there is no barrier to cross-breeding, or when the usual barrier breaks down.

Families with economically important crops often show low hybridization barriers. Simmonds (1976) gives an overview of the history of major and minor crop plants. It appears that hybridization was a common technique in the breeding history of 31 out of 39 families, and in 70 out of 90 genera, i.e. 75-80 percent of the cases. These are crude figures, but they give nevertheless an impression how common hybridization between crop plants and wild relatives is. After all, population genetics has proofed, that hybridization is a major force in the evolution of species.

Adaptative traits acquired under domestication usually have no selective value in natural habitats, and sustainable genetic introgression from domestic plants to wild relatives is rare. Gene exchange is more known in the other direction, from wild relatives to crops (Newmann 1990).

- Reproductive isolation barriers

Isolation barriers can be divided into two types : 1)external or 2)internal to the plant. see Hadley and Openshaw (1980).

- External barriers

External barriers to genetic interchange between related populations prevent pollen of plants in one population from falling on stigmas of plants in an other. Combination of barriers, such as geographical and ecological or ecological and seasonal (flowering time) are more common than individual barriers and tend to reinforce discontinuity between populations (Hadley and Openshaw 1980).

- *Spatial isolation*

The transgenic crop and the weedy relative must be geographically close to allow cross-pollination. Pollen transfer by wind or insects is limited in distance. Pollination success can as well be influenced by the amount of pollen produced as also by the position above ground of the anthers due to filtering effects of the surrounding vegetation. Spatial isolation is a common control method used by plant breeders during the production of commercial seeds to prevent the crop from being pollinated by unwelcome genetic sources.

Ecological isolation

- Even in the same geographical area, two populations can be genetically isolated by adaptation to different habitats or ecological niches. Such separation may be incomplete because the habitats may meet in certain areas within the ranges of both populations, thus giving them the opportunity to cross. Within these contact zones, F1 hybrids and later generation hybrid swarms may arise and allow gene introgression from one population to another.
- *Phenological isolation*
  Two populations occupying the same geographical area and even the same ecological niches may be genetically isolated by different flowering season. This barrier may also be incomplete due to overlapping flowering periods. Even within a single population there may be diurnal patterns: Effective mating may happen in the morning, whereas the main pollinator activity has been detected in the afternoon (Ashman et al. 1993).
- *Mechanical isolation*
  Entomophilous plants often show barriers in their specialised transfer mechanisms. Often entomophilous plants have one single, highly specialised insect pollinator and their flowers are also highly adapted by co-evolution. These specialised flower-insect relationships result in a very effective genetical isolation. Honeybees often concentrate during foraging flights on certain species with high nectar or pollen yield, which is actually a behavioural process resulting into genetical isolation (Hadley and Openshaw 1980).

- **Internal barriers**

  Internal barriers to genetic interchange between related populations operate through disharmonies between physiological or cytological systems of plants from different populations. They may :

1) prevent the production of F1 zygotes, even if the pollen from flowers in one population falls on stigmas of flowers in the other; 2) produce F1 hybrids that are not viable, weak, or sterile, or 3) cause hybrid breakdown in F2 or later generations.

- *Prevention of fertilisation*
  The prevention of F1 zygote formation, referred to as cross-incompatibility, is caused by disharmony between reproductive tissues of plants from different parents. Pollen does not germinate on the stigma; the pollen tube does not completely traverse the style; or the male gamete does not combine with the egg, even though the pollen tube reaches the ovary.
  The failure of pollen growth and fertilisation is rarely the primary cause of reproductive isolation between related species (Hadley and Openshaw 1980). Disharmonies within the hybrid zygote and developing plant or in F2 segregates from fertile F1 plants are more likely to be the major barriers.
- *Hybrid weakness or inviability*
  Some plant species can be crossed to produce hybrid zygotes, but the F1's are either inviable or too weak to be used by the plant breeder. The causes can be grouped within three categories : 1) incompatibility between genomes of parental species, 2) incompatibility between the genome of one species and the cytoplasm of the other, and 3) incompatibility between the genotype of the F1 embryo and the genotype of the endosperm of the maternal parent. Interactions between the parental genomes probably have a polygenic basis and are difficult to be analyzed.

- *Hybrid sterility*
  Attempts to cross two species may succeed in producing a viable, even vigorous F1, but incompatibility between the parental genomes or between the genome of one parent and the cytoplasm of the other may cause the F1 to be sterile. It is called "chromosomal hybrid sterility" when it is caused by structural differences between the parental chromosomes that interfere during meiotic pairing and disjunction. If the sterility is caused by specific gene complexes, it is known as "genic sterility". If sterility is chromosomal, fertility must be recovered by chromosome doubling (alloploidy).

- *Hybrid breakdown*
  Some interspecific F1 hybrids are both vigorous and fertile, yet give rise to F2 plants that are weak or sterile (hybrid breakdown or genetic disability).

2.1.3. Barriers to pollen dissemination

In perhaps the great majority of cases, cross-breeding fails due to the fact that pollen of one individual fails to reach a stigma of a partner. The distance between individuals may be too great or ripe pollen and receptive stigma may be produced at different times. Such spatial and temporal barriers occur at the intraspecific level, tending to prevent self-fertilisation and thus promote outbreeding. Most plant species are hermaphrodite (each flower contain male and female organs) but in some groups the organs may be separated on different plants bearing male or female flower (dioecism) or on the same plant bearing male and female flower (monoecism). But a few plants may also have hermaphrodite flower and in addition separate female flower on the same plant (gynomonoecism) or on a different plant (gynodioecism). The same combination with male flowers is named respectively andromonoecism and androdioecism. In hermaphrodite species spatial separation of the male and female organs may be achieved structurally within the flower as in heterostyly promoting outcrossing. More commonly, separation of pollen and stigmas on the same flower, inflorescence or individual is temporal (dichogamy), attained by the earlier ripening of anthers and shedding of pollen (protrandry) or the earlier production of receptive stigmas (protogyny).

Sexual reproduction occurs among crops, weeds, and wild plants within the distance that pollination can occur and where the plants are sexually compatible. Because pollen transfer is mediated primarily by wind and insects, the distance within which pollination can occur is affected by wind turbulence, speed and direction and/or the flying range of insects (Regal, 1982). Another factor is the longevity of the pollen itself. Generally, pollen is viable only a short time ; that is, it is capable of fertilising eggs only within a time period of hours or days after being produced.

Of particular importance is the aerial transport of pollen grains. With plants that are pollinated by insects, one approach is to follow the pollinator. Insect such as honeybees and bumblebees are often loyal to a particular variety of flower for a given period of time. Moreover, they prefer short trips between blooms, visiting several flowers on a single plant perhaps, and then flying to a near neighbour, a few meters away at most. Yet because bees do not deposit all the pollen from one plant to the next, genes tend to travel further than appearances suggest (genes travelled twice that distance between two stops of bees) (Young 1989). With plants that are pollinated by wind, most pollen is deposited near to home, but a small amount travels much farther (Young 1989). It should also taken into account that indigenous pollinatior insects often are better adapted to lokal variation in climate than honey bees (Boyle-Makowski et al. 1985.)

2.1.4. Are natural hybrids fit or unfit relative to their parents ?

In evolutionary biology hybridization has been recognized as a major factor in the evolution of plants. According to recent studies summarized in Arnold et al. (1995), hybrid genotypes have not been found uniformly unfit. Pooling of different genotypical classes may lead to erroneous conclusions concerning hybrid fitness. The compilation of Arnold demonstrates that a careful case to case study is necessary in the course of technology assessments.

2.2. Which are the traits modified by genetic engineering ?

An OECD study summarises the introduction of transgenic plants all over the world between 1986 and 1992 (OECD, 1993a). The new characters brought by the transgene can be divided into nine classes :

1. Tolerance to herbicides
2. Resistance to diseases
3. Resistance to virus
4. Resistance to insect
5. Quality characters
6. Flower colour
7. Male sterility and restorer
8. Resistance to stress
9. Resistance to heavy metal

Species	Tolerance to herbicide	Resistance to diseases	Resistance to virus	Resistance to insects	Quality Improvement	Male-sterility and restorer	Resistance to stress	Tolerance to heavy metals
Wheat	yes							
Potato	yes	yes	yes	yes	yes		yes	
Apple				yes	yes			
Grape								
Rapeseed	yes	yes	yes	yes	yes	yes		yes
Maize	yes	yes	yes	yes	yes	yes		
Sugarbeet	yes		yes					

Table 1 : Field trials with transgenic crops (OECD 1993a; Department of the Environment, 1994)

In this table, the genetically modified characters for some important Swiss crops tested in field are shown.

2.3. Which of these modified traits should have an influence on the hybridization process ?

Genetic modification may affect hybrid formation either by changing frequency with which it occurs, or by altering the range of species with which the crop is sexually compatible. Increasingly the evidence suggests that modification has little impact on either factor. In general, plant fertility is maintained after the genetic modification, although there are instances where a particular transformation protocol has produced sterile plants. For example, transgenic maize regenerated from protoplasts is sterile if the protoplasts are transformed by electroporation (Rhodes et al. 1988), but fertile if transformed by polyethylene glycol (Omirulleh et al. 1993). Fertility problems in transgenic plants may therefore be due to particular combinations within the transformation protocol and starting material rather than due to the transformation in itself. It is unlikely that totally sterile transgenics would be of interest as they are unsuitable for breeding purposes. It may be assumed, therefore, that commercial transgenic crop varieties will show similar fertility as non-transgenics. One exception is the crop modified for male sterility. These crops are useful to plant breeders.

One of the most important factor of hybridisation is the movement of the fertile pollen to a receptive stigma. Clearly, pollen production in the crop and the stigma-ripening in the wild relative must be concurrent. If the phenology of a transgenic crop is different from the unmodified form, then the potential for hybridisation will be changed. Several genetic modifications have produced alterations in flower development, but these tend to be changes in structure, leading to morphologically abnormal flowers (Mizukami and Ma 1992 ; Mandel et al. 1992) rather than the production of normal flowers with different flowering times. These experiments were done with genes known to be involved in flower development or promoters only activated in floral tissue. Up to now, transformation does not seem to alter flowering times. However, as flowering time is a multigenic trait there is a possibility that a transgene may integrate into and disrupt the expression of a gene involved in flowering-time determination. Assuming simultaneous flowering, the extent of pollen transport will determine the potential for crop pollen to land on the stigma of a wild relative. Pollen flow, in most cases, describes a highly leptokurtic distribution from the source plant, with most grain moving less than 2 m in herbaceous plant (Levin and Kerster, 1974). Recent work confirms this for transgenic pollen (Tynan et al. 1989; Umbeck et al. 1991; Morris et al. in press). However, it is misleading to conclude from these data that hybridisation between crops and wild relatives can only occur over short distances. Even in case a hybridisation over long distances takes place only rarely, it may produce a new, viable founder population.

Pollination is a major determinant factor for the hybridisation rate, but the types of hybrids are determined by interspecific incompatibility mechanisms. The genetics of these mechanisms are still not clear. Unless interspecific incompatibility is controlled by one or a small number of genes, the transformation process is unlikely to alter the cross-compatibility of the plants following transformation. Transformation of a self-compatible species with an S-allele involved in self-incompatibility mechanisms has been achieved, but the transgenic plants became self-compatible (Toriyama K. et al. 1991). Introduction of an incompatibility system into self compatible species will be useful to plant breeders, but it is not clear whether self incompatibility is involved in interspecific incompatibility, and, consequently, such modifications can be expected to have little effect on interspecific hybridisation.

Another important question is whether the relation between pollinators and plants, so important for insect-pollinated species, will be modified in transgenic plants. In France, the group of Pham-Delègue studies this problem with transgenic oilseed rape. First the new proteins synthesized by the transgenic plant should be toxic for bees. The non-toxicity of the chitinase has been proved (Pham-Delègue et al. 1992). Could the character of a transgenic plant modify the activity of the insect ? The same group demonstrated that the foraging time was shorter on a transgenic plant than on a non-transgenic one. However, they concluded that transgenic oilseed rape has no negative effects on foraging bees under controlled conditions. They need to repeat the experiment in the field (Grallien et al., 1995). Similarly, Paul et al. (1991) found that there are no differences in the range of animals and the frequency of visits between modified and non-modified tobacco plants.

Insects like bees are attracted by light of wave length between 300 and 650 nm (Dumas 1984). This includes UV but excludes the red. A change in the flower colour could disturb the attractiveness of the flower for the insects and change cross-pollination rate for insect-pollinated plants.

In an other point of view, genetic engineering may be favourable to the hybridisation process. Studies regarding Medicago sativa treated by a pesticide (dimethoate) clearly demonstrated that this pesticide is found in the pollen and the nectar at a very low level but even at a low level this pesticide is toxic for the bees (Dumas 1984). This has an effect on the entomophilous pollination. Plant resistant to diseases will not be treated with pesticides, so that the efficiency of pollination for entomophilous plants could be modified.

2.4. Problems

The first problem is that hybrids are not always morphologically detectable (hybrids between cultivated and wild lettuce for example (Frietema de Vries 1994) or for *Humulus lupulus* (Newmann 1990)).The second problem is that there may exists genetic variation in the ability to hybridise with other species (difference between genotypes (for example lettuce (De Vries 1990) or Brassica (Becker 1951)).

Small populations are particularly vulnerable to gene flow from a larger population of a related plant than large ones (Ellstrand 1992). If a rare species receives more pollen from a crop than its own kind of pollen, either outbreeding depression or genetic assimilation can be an important problem. Depending on the parent, hybridisation can drastically reduce fitness. The decrease can be manifested as early as reduced seed set or as late as the production of hybrids with reduced vigour or even later as hybrid sterility (Ellstrand 1992 ; Grant 1981 ; Levin 1978). Genetic assimilation is an alternate problem if hybrids are fully fertile and vigorous. The substantial pollen flow from a common crop could dilute the genetic integrity of a rare natural species until the rare form was effectively assimilated into the crop species. In essence, the rare species can be hybridised out of existence.

Another potential adverse impact, likely a rare event, concerns the capacity for a transgene to be reintroduced into subsequent crops from wild/weed populations. The significance of this event would depend on the nature of the transgene product, whether the transgene is expressed, and the fate of the crop, that is, whether it was to be consumed by humans or animals or used for some other purpose.

2.5. Conclusion: Gene flow via pollen

The significance of gene flow via pollen is determined by the extent to which 1) crosses between crops and wild/weedy relatives produce fertile hybrids in which the gene(s) are expressed and 2) the transgenes are retained in a population.

Factors that influence the likelihood of successful crosses between crops and relatives are : first, crop and relatives must grow in a proximity to one another ; that is, within the distance that viable pollen is dispersed. Subsequently, the relative and the crop must be compatible and must cross and produce viable, fertile offspring in which the transgene is expressed.

The amount of empirical data defining the sexual compatibility of crops and relatives varies widely from crop to crop and from relative to relative.

At one end of the spectrum are crops like maize and soybean that have no sexually compatible wild/weedy relatives in Switzerland. For these crops, the likelihood of gene flow into wild populations as a result of use in Switzerland is virtually nil. Elsewhere in the world, particularly in areas where wild/weedy relatives are abundant, such as the centers of diversity and origin of a crop, flow of transgenes to wild relatives is almost certain to occur. At the other end of the spectrum are crops grown in close proximity to sexually compatible wild relatives. In this category in Switzerland, we found plants such as alfalfa, ryegrass, fescue. In these situations, if crop pollen is released at the same time that the compatible relatives are blooming, transgenes will almost certainly enter the wild/weedy population. Between these two extremes are crops that are grown near wild/weedy plants that are not close relatives but may have some degree of cross compatibility. This is the case for potato and oilseed rape as examples.

Results of outcrossing experiments between cultivated crops and related wild species confirm data already available in the literature (Giddings 1995). This suggests that the transgenic nature of the plants does not influence their capacity for outcrossing. Relevant information on sexual compatibility may be available in the plant breeding literature, particularly for the major crops. For many other species, the level of sexual compatibility is unknown. In Switzerland, a considerable amount of information on the identity, distribution and sexual compatibility of native and introduced relatives of many crops is lacking. It is difficult to utilise the results of crosses made in foreign countries as it is well known that the genotype of the wild relatives may be different and influence the result of a cross (De Vries 1990; Becker 1951).

Transgenes that are most likely retained in a population of wild/weedy relatives are those that enhance fitness, that is, confer an advantage strong enough to promote the survival of the transgenic plant over the competitors in the wild/weedy population. So far, transgenes engineered into crops differ widely in their likely adaptative advantage. For example, genes that provide tolerance to environmental stress - pathogens, insects, extreme temperature and drought - are thought to be more likely to have an adaptative advantage than genes that alter nutrient composition or confer to male sterility. But since wild/weedy plants exist in various habitats under a range of climatic and biological conditions, it is difficult to predict which traits will provide the competitive edge in a particular environment for any specific population. Neutral transgenes - those neither beneficial nor detrimental to the plant - may also become established and spread under various circumstances :

1. High rate of gene migration (Ellstrand and Marshall 1985)
2. Genetic drift in small populations (Ellstrand et al. 1989)
3. Linkage on a chromosome to other genes that confer an advantage.

Finally, transgenes may be retained, even if they are disadvantageous, because they are recessive (Curtis 1983) or because of genetic drift.

- Genetic engineers have an enormous pool of new genes so they can add more genes with harmful potential than can traditional breeders.
- Novelty and power of transformation technology suggest that its outcome will be not less predictable but create other potential risks compared to the result of traditional breeding
- Many of the transgenes being transferred into crop produce traits which appear obviously advantageous to plants. New traits such as resistance to disease, cold, or herbicides, would enable weeds to overcome ecological limits on population growth. In contrast, many of the traits established in most crops by traditional breeding tend rather to reduce than to enhance the fitness of wild relatives (Ellstrand 1988; Regal 1992). Traditional breeding often selects for types of genes that are agronomically important but interfere negatively with natural adaptability.
- The capacity to combine genes from totally different organisms is an unparalleled occurrence in evolution.

The transgene could also escape from a crop via seeds (Gliddings 1995): The cultivated plant could receive some pollen from the weed, then some hybrid seeds could be produced and some of those seeds could be dispersed.

The importance of a crop/wild hybrid system would depend to a large extent on its comparative competitiveness in the environment to which it became dispersed. It has already been mentioned that many researchers consider that such plants would be ill adapted for competition with existing weeds. This however is probably an oversimplified and short term view. As long as the plants are reasonably fertile it is quite possible that, after a few generations of seed production, types would arise with better adapted combinations of genes. Some pressure in favour of the hybrid type such as a disease epidemic after inheritance of the appropriate resistance, might accentuate the process. Furthermore chromosome doubling among hybrids is known to have turned sterile weeds into aggressive and fertile pests (Gliddings 1995).

It is improbable that genetic modification will change the rate at which crops hybridise with wild relatives, or the range of species with which they are sexually compatible. As a whole, the authors hope that discussion on risk assessment of transgenic crop production will produce a thoughtful feedback to problems with classical breeding, which have been existing for a long time.

3. Weediness

3. 1. General Introduction

Agronomists and ecologists use the terms *weed* and *weediness* in different ways, and this is often a source of misunderstanding, especially in discussions concerning the release of transgenic plants. For agronomists, the problem of weediness is solved if the aggressive weed can be removed from the agrosystem by means of adapted measures. They are not interested in its behaviour outside of agrosystems, i.e. in (semi-)natural habitats. From the ecologist's perspective, invasions of weeds into (semi-)natural plant communities are risky. Highly competitive invaders are able to disturb the pattern of species. As a result, weak, often rare species may be eliminated. In the worst case, the invader succeeds in occupying the entire surface as a near monoculture.

Regarding weed problematics, gene flow from crops to closely related weeds is essential. In the following example, we concentrate on a crop with a transformed disease resistance. If this resistance gene gets into a closely related weed population by means of hybridization and introgression, it may increase the competitiveness of resistant individuals. Such plants may evolve into a problematic weed which is difficult to be controlled in agrosystems. In case the new weed is able to grow outside agrosystems, it may cause modification in (semi)-natural plant communities as mentioned above.
The disease resistance may reveal to be a decisive factor for competitiveness enabling the species, which has so far been retained by disease attacks to spread, thus upsetting the pattern of species.

But even crops themselves may cause a weed problem as studies on *oilseed rape* have shown (Schlink 1994, Schönberger et al. 1991). Nowadays, volunteering oilseed rape (*Brassica napus*) has to be controlled by means of an adapted crop rotation system and tillage techniques, sometimes combined with herbicide applications. Furthermore, feral populations can often be observed in disturbed habitats outside agrosystems. How long such a population can survive needs to be checked. In addition, its potential for invading natural plant communities has to be analysed. (These questions will be studied in the framework of the SPP project of Biotechnology 1996-99 by F. Felber, R. Guadagnolo, J. Keller Senften, P. Rufener Al Mazyad and D. Savova).

By means of an appropriate long-term monitor system observing potentially problematic weeds and their potential (natural) habitats, changes in the pattern of wild species have to be detected at an early stage. Also certain crops, such as oilseed rape will have to be included in the future monitoring system. It is an illusion that problematical weed types can „easily“ be eradicated (see Hartmann et al. 1994). Therefore, early detection of such weed types is essential.

3. 2. Definitions

3.2.1. What is a weed ?

For an extensive review of definitions, concepts and ecological characterisations of weeds and of the anthropogenic flora see Lambelet-Haueter (1990, 1991) as well as Holzner (1982) and Oka and Morishima (1982). The current literature reflects the fact that various concepts coexist and that there is not the one classical approach generally accepted.

Lambelet-Haueter (1990) divides weed definitions up into popular, economical and ecological concepts whereas Holzner (1982) groups them similarly into subjective and ecological ones.

Popular as well as *subjective concepts* define weeds as plants growing in the wrong place, causing damage, being of no benefit and suppressing cultivated plant species.

Economical concepts reflect the view of agronomists who concentrate on the phenomena in agrosystems. Competition between crops and weeds, which reduce yield production is central to the definition. Thereby, the damage aspect is stressed. A weed problem is solved as soon as the plant no longer creates considerable damage in the fields, a state which is reached by means of adjusted weed control (crop rotation, tillage, herbicide application).

In contrast to the previous concept, *ecological definitions* include habitats outside agrosystems colonised by weeds. The usual preference of weeds for anthropogenically disturbed habitats is stressed. They include cultivated fields and gardens which are artificially kept open as well as disturbed areas on road sides, recently built artificial slopes and others. An aggressive weed can cause damage not only in agrosystems but also in (semi-)natural plant communities by outcompeting weak species.

Following Holzner (1982, p. 5), it is sometimes difficult to call a plant a weed because one and the same species may be considered in some parts of its area as a harmless component of natural vegetation, in others as a weed and again in others, even as a useful plant species.

Williamson (1988) pays attention to the fact that 17 out of 18 most feared „World's Worst Weeds" (Holm et al. 1977) are also cultivated. The list was integrated into the German technology assessment by Sukopp and Sukopp (1994): *Cyperus rotundus, Cynodon dactylon, Echinochloa crus-galli, Echinochloa colonum, Eleusine indica, Sorghum halepense, Imperata cylindrica, Portulaca oleracea, Chenopodium album, Digitaria sanguinalis, Convolvulus arvensis, Avena fatua, Amaranthus hybridus, Amaranthus spinosus, Cyperus esculentus, Paspalum conjugatum, Rottboellia exaltata*.

Except for *Portulaca oleracea*,the above mentioned species are not cultivated in Europe.

3.2.2. Weed characteristics / weediness

Weeds are perfectly adapted to live conditions in anthropogenically disturbed areas. Thus, surviving strategies of weeds are so various that any list of weedy characteristics remains incomplete, even the famous one of Baker (1967, 1974, p. 4). He lists the following characteristics of an ideal weed:

Ideal weed characteristics (after Baker, 1974):

1. Germination requirements fulfilled in a broad range of habitats
2. Discontinuous germination (internally controlled) and great longevity of seeds
3. Rapid growth through vegetative phase to flowering
4. Continuous seed production for as long as growing conditions permit
5. Self-compatible but not completely autogamous or apomictic
6. When cross-pollinated, unspecialized visitors or wind utilised
7. Very high seed output in favourable environmental circumstances
8. Produces some seed in wide range of environmental conditions; tolerant and plastic
9. Adaptations for short- and long-distance dispersal
10. If a perennial, vigorous vegetative reproduction or regeneration from fragments
11. If a perennial, brittleness, so not easily drawn from ground
12. Has ability to compete interspecifically by special means (rosette, choking growth, allelochemics

When applying Baker's concept of the ideal weed, we have to consider that in reality, a weed never possesses all the characteristics of the list, consequently we have to speak about a weed-*syndrome* with even additional characteristics.

For a discussion of Baker's characters by means of statistical analysis see Williamson (1993).

Closely related species may show dissimilar behaviour. According to Williamson (1993), in Britain there are native, invasive and pest-like *Impatiens* species . In a detailed study of their biology, he shows that they have a range of ecological behaviour parallel to relatively small morphological differences. To predict and detect pest status, a well-defined monitoring system is needed. (see chapter 4.6.4.).

The only attribute which all weeds might have in common is a marked plasticity enabling adaptation to continuous environmental changes.

3. 3. The origin of weeds

For summaries of the origin of weeds see Zoldan (1993) and Rauber (1977). Rauber (1977) lists the following pathways through which weeds evolve even today:

1. wild plants evolve into weeds
2. hybrids between crop and wild relatives evolve into weeds
3. crops evolve into weeds

Especially point two and three are important for this study.

For weed evolution, co-evolution of the crop-weed complex is essential. Pickersgill (1981, p. 378) shows the following phylogenetical relations in crop-weed complexes:

Three different evolutionary relationships between crops and their weedy relatives are possible.
- Firstly, the weed may have been domesticated to produce the crop.
- Secondly, the weedy races may be derived from the crop.
- Thirdly, crop and weed may have diverged simultaneously from a common ancestral wild population.

Rye is a well-known secondary cultigen having evolved from the perennial diploid weed Secale montanum Guss.

3.4. Crops running wild

According to Sukopp and Sukopp (1994, p. 5) who follow the definition of naturalisation by Thellung (1912), a crop has successfully run wild if a crop species usually showing domestication characteristics has developed a range of characteristics of a wild indigenous species, i.e., if it grows and reproduces naturally without the care of man, if it appears more or less frequently and continuously in suitable habitats and if it has succeeded in surviving for a number of years (even years with extraordinary climatic conditions). For details see chapter 4.3.
Having occupied an ecological niche, a plant may develop poorly and may be eliminated after a short time, it may be integrated inconspicuously in the existing species pattern or it may turn out to be an aggressive, competitive weed (often only after many years of adaptation).

In its range of cultivation within Central Europe, cultivated oilseed rape (*Brassica napus*) frequently invades segetal and ruderal habitats. Rich (1991) lists the habitats of feral oilseed rape for Great Britain and Ireland:

> A common yellow crucifer of roadsides, waste and cultivated ground, docks, cities and towns, tips, arable fields, riverbanks etc. Widely cultivated for seed oil or as a forage crop and consequently casual or naturalised wherever oilseed rape is grown on the British Isles.

3. 5. Crops showing weed characteristics

Following Schlink (1994), almost all crops are able to appear as volunteers in the subsequent culture after remaining in the field by harvest loss. The trend towards short term crop rotation systems poor in species has stimulated the spread of volunteers. As well as weeds, volunteers of various crops compete with the cultivated crop for growing factors. Furthermore, they are a potential intermediate host of pests in crop rotation systems.

3. 5.1. Case study *Brassica napus*, oilseed rape

This phenomenon is presented below showing data of oilseed rape (*Brassica napus*) by Schlink (1994) who extensively studied its *weed characteristics*. Concerning germinating ecology, *Brassica napus* has all requirements to establish itself as a „wild plant“ in an agrosystem with tillage or in a ruderal habitat. Furthermore, in its growth behaviour and in its high potential of reproduction, oilseed rape resembles segetal weeds and thus represents a typically competitive weed. In crop rotation systems including oilseed rape, the „unidentified“ volunteering rape is capable to pass through all developmental stages up to seed maturity. Seed loss before and during harvesting encreases the seed stock in the soil even by seeds produced by volunteers. In this way, selection of enduring genotypes in the field is possible. Simultaneously, the seed stock in the soil is constantly enlarged by new genotypes due to rapid development of new varieties of oilseed rape.

Excerpts from a manual for experts in cultivation of oilseed rape in Top Agrar, Rapsanbau für Könner 1991:

> Oilseed rape germinates like a weed. Under favourable conditions, it appears four or five days after sowing (Schönberger and de Vries 1991, p.23.).
> Volunteering oilseed rape is problematic in the subsequent culture, whereby lost seeds may germinate over a long period of time and therefore may cause problems. Due to application of growth hormones, control of volunteering oilseed rape in cereals is no problem [per se]. But volunteering oilseed rape can become a leading weed, especially in dry zones. Because of its long germination period, its high competitiveness and difficulities in harvesting, herbicide application after germination will be necessary even if no weeds are present. Control of volunteering oilseed rape in culture of oilseed rape is very difficult. Therefore stands may vary greatly in their density which is negative for survival in wintertime and for quality, favours pest growth and depression of ripe plants (Blanck 1991, p. 98).

Soil seed bank:
The ability of seeds to survive in the soil for a long period of time is caused by dormancy, germination biology and ecology, even under changing environmental conditions.

Following Zohary (1992), the spread of seed germination over two or more years is a common adaptation particularly of annual species. Partitioning of seed germination yield over two or more years is an effective device to buffer the otherwise crippling effects brought in by climatic fluctuations. But such allocation does not serve just to evade disasters. It also acts as a balance which buffers the selection in any particular year, and prevents extreme annual shifts in the genetic composition of the population.

According to Schlink (1994), crop breeding generally selects well-germinating genotypes. Especially in the breeding of winter oilseed rape, which can be sown just after harvesting, high germination rates of fresh seeds might have been a secondary breeding goal. In contrast, seeds of oilseed rape are able to survive for a long time in the soil what has been proven by model experiments using four different varieties. They have shown surviving rates of over 70% for a period of 1,5 years and of almost 60% after five years of exposition in the soil. These rates usually are met only by weeds. The surviving seeds of oilseed rape were sensitive to light which is characteristic for wild species that are adapted to segetal and ruderal habitats. Furthermore, the surviving seeds in the soil showed changes in their germination readiness due to a dormancy cycle induced by seasonal shifting of soil temperature. Such a survival strategy is well-adapted to ecological conditions in temperate zones. It is a typical characteristic of wild plants (Schlink 1994, pp. 136-138).

According to Schlink (1994), the strategy of eradicating volunteering crops in agrosystems, as it was followed some decades ago, is not reasonable, due to the fact that the supply of fresh seeds to the soil seed stock is guaranteed by the cultivation of the crop itself.
Even if seed loss during harvesting could be prevented, not all sown seeds would germinate under particular conditions as certain genotypes would develop a secondary dormancy and would therefore be added to the bank of dormant seeds.

3.5.2. Case study *Beta sativa*, sugar beet

In contrast to biennial sugar beet, in weedy annual forms specific alleles cause early development of stems and inflorescences already in the first year (Rauber 1977). Feral, early shooting and blooming sugar beet cause certain problems in Switzerland. (Weedy hybrids between sugar beet and sea beet have not yet been found in Switzerland, they seem to be restricted to the Atlantic part of Europe). In case of development of ripe seed before the harvest period they can produce feral seed banks persisting for many years. Offspring of these seeds show again strong tendency to early shooting, therefore being a serious weed problem in sugar beet fields. According to Bartsch (1995) annual forms can be a result either of vernalisation during cold springs or evolve through introgression of dominant genes causing annual forms from wild populations. This would contradict the opinion of Rauber previously cited. Compare also chapter 3.8. on genes for weediness.

3.6. Reversion of crops to wild types

There is no reference to a case where crops would have totally been reverted to their wild type or where they would have lost all domestication characters (Sukopp and Sukopp 1994). Centuries or even millennia of domestication obviously cannot be taken back easily.
According to the NRC Report on Field Testing (1989), domesticated crops, such as wheat, maize and soybean, have been genetically modified in traditional breeding to such an extent that they can no longer compete effectively with wild species in natural ecosystems. These crops are unlikely to revert to a weedy condition upon further genetic modification. Some less domesticated crops as forage grasses [and oilseed rape] are more likely to revert to a weedy condition.

However, the example below shows that a single gene change may be sufficient to revert a crop to a wild type. This has been shown by a photograph of Schwanitz given by Rauber (1977) with the example of Maize, which turned into „corn-grass“ having a much smaller size in stem and leaf.

In addition, Sukopp and Sukopp (1994) as well as Bartsch et al. (1993) noted that in case of cultigens which have a low degree of domestication, one mutation can cause the weedy form which then successfully spreads. The loss of spikelet spindle toughness of cereals, for example, is sufficient for regaining the ability to spread diaspores (e.g. *Avena*).

Another example is *Avena sativa* showing the fatuoid mutant: Loss of a combination of genes which suppress awn, pubescence and easy dehiscence of caryopses transform *Avena sativa* back into nearly wild plants (Roesler 1969 in Rauber 1977).

3.7. Weeds evolving from hybridizations between crops and related wild species

For details see chapter 3.3.
The NRC Report on Field Testing (1989) gives a list of case studies of crops becoming weeds after hybridization with wild relatives: Weedy beets in Western Europe; *Secale cereale* in California, where a weedy rye probably derived from a cross between *S. cereale* and *S. montanum* is leading to the abandonment of rye cultivation; Squash (*Cucurbita pepo*) -

important in the Southern United States, already genetically modified; "Hybrid Grain Sorghum" and others.

3.7.1. Brassica napus, oilseed rape

It is a biological fact that genes will escape from transgenic oilseed rape into the gene pool of *Brassica napus* which contains not only *B. napus* but also its relatives *B. rapa* subsp. *campestris* (L.) Clapham *(= B.campestris* auct.*), Raphanus raphanistrum* and other species.

Introgression of genes of oilseed rape (*B. napus*) in natural populations of *Brassica rapa* subsp. *campestris*:
In an ongoing research program (Group of R. B. Jörgensen at Risö, Mikkelsen et al. (1995), the behaviour of transgenic *B. napus* (herbicide tolerance, insect or fungal resistance) and *Brassica rapa* subsp*. campestris* is studied in natural habitats by screening populations over several years using non-destructive methods. Other populations are harvested yearly to check biomass and seed production. Their experiments revealed transgene flow from oilseed rape to *Brassica rapa* subsp. *campestris* and introgression by backcrossing in the test field.

Jörgensen (1993) and Jörgensen and Andersen (1994) underline the importance of weedy *Brassica rapa* subsp. *campestris,* a common weed in Northwestern Europe. (In Switzerland, re-discovered in 1995 by Pia Rufener Al Mazyad as a common weed in traditional mountaneous agriculture systems*).*

Brassica rapa subsp*. campestris* possesses many agronomically important characters (e.g. yellow seed colour, pathogen resistance, cold tolerance) that are demanded in the breeding of *B. napus*. This together with the good cross-compatibility makes the species an attractive gene resource.
With efficient agricultural practice the wild form of *Brassica rapa* subsp*. campestris* is almost exclusively found as a weed in oilseed rape fields where herbicide elimination is not applicable. However, herbicide treatment in oilseed rape fields will be possible with the introduction of genetically modified oilseed rape with genes for herbicide tolerance. These genetically modified varieties are already in field testing and will be marketed within few years. When this happens the wild form of *Brassica rapa* subsp. *campestris* could be endangered.

In addition, weedy *Brassica rapa* subsp. *campestris* and its hybrids with oilseed rape could be disseminated with certified seeds. Herbicide tolerant oilseed rape might induce the evolution of a new weed (Jörgensen and Andersen 1994, p. 1635):

> As the gene for herbicide resistance is likely to be transferred to *Brassica rapa* subsp*. campestris* by hybridization and backcrossing, the use of this herbicide strategy will be inapplicable after a few years. Like many other weeds, *Brassica rapa* subsp. *campestris* is characterised by seed dormancy and longevity of the seeds. Therefore, *Brassica rapa* subsp*. campestris* with transgenes from oilseed rape may be preserved for many years in spite of extermination efforts. *Brassica rapa* subsp. *campestris* with other types of genes transferred from *B. napus* might affect natural ecosystems as well as the agro-ecosystem.

For an overview of additional hybridization experiments between oilseed rape and close relatives see chapter 2. hybridization and Sukopp and Sukopp (1994).

3.7.2. *Beta vulgaris,* Sugar beet

Weed-beets, hybrids between sugar beet (*Beta vulgaris* ssp. *vulgaris*) and the wild type seabeet (*B. vulgaris* ssp. *maritima*) are more aggressive in culture than the wild type. This has been shown since the seventies. For an overview see Madsen (1994) and Pickersgill (1981), as well as Sukopp and Sukopp (1994).

After Bartsch a main source of weediness after gene introgression is pollen transfer from *Beta vulgaris ssp. maritima* into seed production fields of *Beta vulgaris ssp. vulgaris* in the Netherlands and Northern Italy.

Fredshavn and Poulsen (1993) studied differences in the competitiveness of the *Beta*-complex when using transgenic *Beta*. There was no enhanced competitiveness observed. For a discussion of transgenic *Beta* see 4.4.4.3.

3.8. Genes of weediness

There is space for only a few examples for weediness genes here. Due to the fact that weeds have a large variety of characteristics, as is shown in paragraph 3.2., no definitive list can be given. See also chapter 4.3.1.

Lupi (1995, according to OECD 1993b) states in his BATS report that according to Baker (1974), weediness is a multicharacter attribute and the addition of one gene is unlikely to cause a crop to become a weed. In contrast, Fitter et al. (1990) and Williamson et al. (1990) suggest that the alteration of one gene may indeed be enough to change a crop into a weed. If a crop species has very few weedy characteristics, the addition of one or a few genes would be unlikely to cause the crop to become a weed problem. Special attention might be warranted where the crop has weedy characteristics or the added genes might be expected to improve the crops competitive ability in natural or agricultural ecosystems.

Also in the above cited case of *Beta vulgaris ssp. vulgaris* Hoffmann et al. (1970) in Rauber (1977) state, that cultivar and weedy relative are identical except for one single allele B/B+.

3.9. Herbicide tolerant weeds

For most of the existing studies, comparisons between transgenic and non-transgenic crops, which have been done in view of weediness, are based on herbicide tolerance. One of the reasons for this is that herbicide tolerance serves as easy marker gene in a well defined artificial system, since herbicide treatment is not done outside agricultural systems.

3.9.1. Natural herbicide tolerance in wild species

Gressel and Kleifeld (1994) studied a case of a spontaneous mutation causing herbicide tolerance in *Brachypodium distachyon.*

> It was a surprise when a relatively rare, innocuous grass species, *Brachypodium distachyon* (L.) P. Beauv., appeared as a monoculture along simazine-treated roadsides in Israel over ten years ago, having target-site resistance to s-triazine-type herbicides (Gressel et al., 1983). The wild type was sensitive to triazine herbicides. There is a strong possibility that Brachypodium seeds came with the road foundation material, thus bringing the large quantities of seed needed for selection. Sterilant levels of simazine were used along the roadsides selecting for the rare resistant individual(s). Seeds of such individual(s) rapidly spread due to vehicular movement, as well as run-off, resulting in hundreds of kilometres of roadsides being covered by this weed. The road authorities continued to apply simazine; nothing but *Brachypodium* could grow until seven other grass species and *Amaranthus blitoides* (covered with *Cuscuta*) evolved resistance to triazines. These other weedy species were all indigenous to the nearby agroecosystems. Simazine-resistant *Brachypodium* could not compete with the truly weedy species and was displaced, becoming rare. The road authorities then began using other herbicides (especially diuron), which easily decimated the *Brachypodium*. One must go back to the sandstone hills, its niche in the wild, to find it. It remains a weed only in some olive orchards and industrial sites where simazine is still used as a sole herbicide. These are all on poor soils, similar to some extent to its wild home, and the other simazine-resistant grasses are taking longer to displace the *Brachypodium* in such habitats.
>
> Implications
> We believe that these case histories indicate the following:
>
> 1. Wild species can acquire genes for herbicide resistance. In the documented cases the acquisition was by selection; it could occur in other species by cross-pollination with crops.
> 2. Such selections can probably only occur in the rare instances when a large population of the wild species is brought into contact with a herbicide or with a related resistant crop species. Nevertheless, we do not know of instances where crops have conferred any of their natural herbicide resistance's on wild relatives. With Brachypodium, selection was possible after moving seed from the wild to a treated area. The same could also occur with other wild species when virgin land is brought into cultivation. It may thus be advisable initially to use mechanical weed control to lower the level of wild species in a new agroecosystem.
> 3. Genes for herbicide resistance can temporarily elevate wild species to weed status.
> 4. Resistant wild species will remain weedy until more typical weed species evolve resistance and displace the wild species or until the selective herbicide chemistry is replaced by a different chemistry.
>
> Thus, there seems to be little risk of a wild species remaining a weed for long periods.

It seems logical to conclude from the example of *Brachypodium* that if a transgenic crop transfers herbicide resistance genes to a wild relative, that species will most probably be a weed for only a short period.

Critical comment of the authors:
Generalisation from that one case to any plant species and any type of transgene as it is proposed by Gressel and Kleifeld (1994) plays down the risks and is not acceptable for a serious risk assessment.
We do not know enough to back up such generalisations. In some additional caveats the authors themselves do relativise the above made permissive statements. They admit that there are herbicide resistances as the mentioned simazine resistance which reduce considerably the competitiveness of the new weed, while others in contrast do not.
Still it is remarkable, that in this exceptionally well documented case with an unwillingly clear-cut experimental set-up there are mechanisms of competition described, which indicate, that in certain cases the worst case scenario of an escaping resistance gene turns out to be by far not so dramatic as stated in most papers critical to genetic engineering.

3.9.2. Herbicide tolerance in wild species induced by hybridization with herbicide tolerant transgenic crops

Madsen (1994) tested the competitive ability and growth behaviour of a hybrid between seabeet (*Beta maritima*) and transgenic sugarbeet (*Beta vulgaris*) with a glyphosate resistance. She tested in a field experiment whether the hybrid had a higher biomass and a higher competitive ability than the non-transgenic parental types. The hybrid did not produce more biomass than sugarbeet and the competitive ability of the hybrid did not exceed the expected level of a non-transgenic hybrid between sugarbeet and seabeet. Considerations for the release of herbicide tolerant crops have been published by Bainton (1993). The author concludes that, although there are no grounds for major concern, the Ministery of Agriculture, Fisheries and Food of the United Kingdom should remain alert to adverse developments and be ready to investigate any matters to which the Advisory Committee on Releases to the Environment draws attention.

See also the case story of *Avena* in chapter 4.4.4.2.

In a general discussion of herbicide resistance evolving herbicide resistant weeds, Madsen (1994) concludes that during herbicide applications, selection pressure from e.g. glyphosate is posed on the population privileging herbicide resistant types what should be prevented by crop- and herbicide-rotation. Recalling the case study of *Brachypodium* by Gressel and Kleifeld (1994), for certain herbicide types the developed resistance of a weed will only be problematic during herbicide application.

Regarding (semi)natural habitats, Crawley et al. (1993) and Timmons et al. (1996) acclaim that a herbicide resistance outside the arable land does not provide an advantage to a wild relative, because there is no selection pressure in favour of herbicide resistance in natural habitats. For more details see chapter 4.4.4.1.

Sukopp and Sukopp (1994) add that there are other odds against a rapid spread of crops in natural habitats: Long term observation experience of traditional weeds of agricultural systems show that these species are often nicely confined to areas strongly influenced by man. Massive application of herbicides has led to the development of numerous herbicide resistant weeds up to now.

3.10. Enhanced weediness in transgenic crops?

In their famous field trial, Crawley et al. (1993) showed (see also chapter 3.5.1., 4.2., 4.4.4.1) that the analysed transgenic varieties of oilseed rape were slightly less competitive than traditional ones. Considering population biology, the analyses can be criticized in the way that just mean values are discussed. Weber (1995) demands in her critical discussion of Crawley et al. (1993) that risk problems accessible to empirical verification should actually be approached empirically. Also she emphasises that the spectrum of methods should be widened.

She presents the following long-term *scenario* for transgenic oilseed rape which seems to be compatible with the results of the experiments carried out by Crawley et al. (1993) (p. 121-122):

1. Transgenic oilseed rape may change to be more invasive through recombinant characters or through position effects or through selection effects by herbicide application to herbicide tolerant crops or even through further evolution.
2. Transgenic oilseed rape may establish itself in disturbed places, from where it could invade undisturbed habitats.
3. Transgene oilseed rape individuals could develop enhanced competitiveness and invade thus undisturbed habitats.

Again we have to emphasise that the above comments of Weber are purely hypothetical and first hints of other authors show that these worst case scenarios do not apply for this case.

Sukopp and Sukopp (1994), p. 67 stated that:

> After three years running time the following results can be seen (Crawley et al. personal communication and Crawley et al. 1993): Transgenic and non-transgenic crops (oilseed rape, potatoes, maize) have the same competitiveness outside agrosystems. They hardly can persist more than one generation. In no case sexual reproduction has been observed.

See also the discussion about *Brassica napus* in chapter 4.3.2. and 4.4.4.1.

3.10.1. Analogy from conventional plants

Since there is no long term monitoring on transgenic crops existing which concentrates on weediness in all aspects, scenarios must remain speculative.

According to Fredshavn et al. (1992), the environmental consequence of releasing transgenic plants to unconfined conditions depends on the changes in survival rate, growth behaviour and hybridization possibilities caused by the transformation.
Survival rate depends on the growth conditions: soil type, water and nutrient supply and plant cover. Crucial for invasion of natural habitats is the establishment period immediately after the seed has germinated („the child mortality“). Later the competitiveness of the plant determines the success as an invader. Fundamental changes in growth behaviour may allow the plant to invade new habitats not formerly occupied by the non-transformed genotype, but more likely, the growth behaviour is only slightly modified, and the transformed plant is limited to the same habitats as the nontransformed genotypes.

Such phenomena concerning sensitive developmental phases should be considered when planning a long term monitoring system.

From the literature, Madsen (1994) concludes that there is no evidence that herbicide tolerant crop plants should become weeds, unless they already possess the traits for weediness, and if only one herbicide is used consecutively in several crop rotations for a longer period of time.

Long before transgenic herbicide tolerant crops have been developed, Rauber (1977) pointed to the possibility of negative consequences: The following scenario developed by Rauber is still valid today.

> New developments are made possible with the availability of modern herbicides: Their impact lacunas produce ecological niches for resistant populations. A possible future problem is that new weeds could emerge from hybrids from crops and their wild relatives (cultivated and wild oat) and also from the crops themselves (sugar beet and weedy beet). In spite of or because enhanced precision physiological and ecological selectivity of future herbicides, it will be more and more difficult to fight these new tolerant varieties. They will have the same genome as the cultivar, except for at least one allele causing weediness. Possibly there will be some future annual weeds,

developing as a perfect mimicry to crops, in this way reaching back to prehistoric times where weeds and crops where still very close and connected through a full range of intermediate forms in fields and seed mixtures.

3.10.2. Is it possible that crops having acquired pest resistance through transformation could turn into aggressive weeds ?

There where no indications in the literature screened for this study.

4. Ecological view

4.1. Some basic thoughts about safety research

4.1.1. Major possible ecological risks of transgenic crops

In a special issue of the Journal of Molecular Ecology (Vol.3, 1994) several authors have summed up the possible ecological risks which are judged to be of major concern:

- Side effects of transgenic products (allergic reactions etc.)
- Invasiveness of transgenic products (crop or cultivar becoming invasive through genetic engineering)
- Invasiveness of the transgene itself (vertical gene flow through hybridization with wild relatives)

Gould (1988a, 1988b) and Thacker (1994) have pointed out that there is an additional major concern to be added:

- Development of pest resistance on plants (in which the transgene codes for a product giving enhanced insect pathogen control (see 4.5.)

Derived from all the facts put together in this study, transgenic crops need not necessarily be a huge risk by reason of their (novel) transgenes artificially transferred to crops.
We should, basically, try to avoid a strict focusing on transgenic crops when considering ecological risks of crops. For hundreds of years mankind has operated with highly artificial non-transgenic crops and cultivars. Many genes (or gene combinations) responsible for various kinds of resistance against insect pests have been brought back to crops by classic breeding methods and subsequently released in large numbers into the field.

In words used normally only for transgenic crops there have been transferred artificially in hundreds of well documented cases new genes into crops, by means of conventional hybridising methods. These newly bred crops have been released in the field without specific risk studies regarding their hypothetically altered ecological characteristics. Position effects, alterations in competitional properties can just as well be a potential risk in new breeds created by classical methods. Certainly we can see a new, more prudent attitude emerging for new crop plants created by classical methods. The molecular understanding of genetics has also created a more thorough discussion about the value of any given genome.

In the course of breeding new cultivars we should apply some safety standards, regardless whether they have been created by classical or modern molecular methods, since unexpected effects can be created by any field release of new crop plants. On the other hand, this broader view reduces to a certain extent anxiety, which often grows into hysteria about the new risks which could be caused by transgenic plants. This statement should not be misunderstood as a poor excuse which might free us from safety considerations in this field. But it will hopefully provide us with an opportunity to create safety standards and safety procedures, which are more realistic and which are not preventing future research and development in genetic engineering.
It should not be denied that novel genes can create novel risks, but there are strong hopes that new procedures of risk assessment should enable us to distinguish between factual and potential risks of vertical gene flow in general.

In our view it is important to listen to specialists of population genetics such as Gabriel (1993), since his conclusions speak against premature generalisations which are based solely on a few and very restricted experiments. He is also opposing a direct comparison between natural mutants obtained through classical breeding and transgenic plants. In his view it is impossible to model in a simple way the full complexity (species, population, ecology, biogeography) of a given crop plant and its wild relatives. Measuring gene flow by means of paternity analysis is shown by Adams et al. (1992). Again, we realise the full complexity of the topic of vertical gene flow.

This is also the view of Regal (1994), who points out basic differences between genetic engineering, where only *genes* are moved and classical breeding, where usually *alleles* are moved around. Still, Regal does conclude, that regardless of the above, many transgenic forms *will* be non-competitive because, (1) the parent organisms were highly modified forms such as extensively domesticated corn to begin with, (2) there may be cases in which the genetic engineering process itself does demonstrably incapacitate the transgenic form ecologically and (3) if the host is the sort of foreign wild species that simply cannot persist without human help under local conditions of inappropriate weather, soil, etc. Biotechnology is unlikely to turn it into a locally ecologically vigorous organism. Still, domestication should not be the miracle key word for safety of any transgenic crop, *a careful long term monitoring should be undertaken in order to determine the factual risks.*

Bartsch et al. (1993) also points out the difficulty of modelling complex ecosystems in urban and agricultural environments. He postulates experiments in confined and open systems within the framework of long term monitoring on the basis of scientific criteria. It is true that from modelling, one can learn a great deal, but findings remain inconclusive. Only in rare cases is there a possibility for true causal analysis. Andow (1994) suggests that mathematical models of resource competition might be useful for identifying categories of plants that either are unlikely to alter community structure or that have the potential for altering community structure.

Well planned experiments with harmless tracer genes which can be screened on their pathway into nature will hopefully get a high priority. These experiments should be accompanied by spatial and multivariate analysis. A recent publication by Kjellson et al. (1994) demonstrates the full complexity of risk assessment research itself: The catalogue contains more than 80 methods for the risk assessment of transgenic plants, assessing competition, establishment and ecosystem effects of future transgenic plants. This volume will be followed by a second volume concentrating on vertical gene flow.

This does not mean that with experiments on a short term basis we will be able to solve all problems.

The dilemma is obvious but inevitable: Only a mass release will bring to the surface all risks and negative effects, as it has always been with the introduction of any new technology. Raybould et al. (1994) also make a plea for a realistic scenario for the risk assessment. One way to minimise potential risks is to study more carefully the natural vertical gene flow of crop plants and their wild and feral relatives. Long term monitoring with an *"end-of-the-pipe"-philosophy* will allow us to conclude on a scientific basis. This strategy implies automatically a case to case study taking into account a regional view.
This is exactly the basic scheme of a research project within the Swiss Priority Programme Biotechnology, Module 5b, which has been supported by the Swiss Government and which is now in its final first phase and which will be continued for another three years (Ammann et al. 1994). The project concentrates on the assessment of a possible vertical gene flow of some 20 non-transgenic crop plants and their wild and feral relatives under natural conditions.

4.2. Is there any difference in the choice of the cultivar or crop regarding risk assessment in genetic engineering ?

Differences in the choice of the cultivar regarding risk assessment in genetic engineering are considerable and are closely related to the degree of domestication. Vertical gene flow is lower in highly domesticated crops such as *Hordeum* and *Triticum* and higher in crops which are close to their wild relatives such as *Brassica napus* or *Medicago sativa*. For a provisional judgement about the consequences for a risk assessment of transgenic crops we refer to 3.5.1, a case study of *Brassica napus* and also to 4.4.4.1-3.

4.2.1. A comparison with the daily mega-experiment of newly introduced genomes

According to Sukopp and Sukopp (1993) there are hundreds if not thousands of new and foreign genomes introduced with trees, shrubs, herbs, microbes and higher and lower animals each year. Many of those survive and can, after years and even many decades of adaptation, begin to be invasive. This trend, overlooked by most and realised and judged to be a true hazard by only a few ecologists, is not evident, since it works slowly but steadily all over the world thanks to human activity in transport and tourism, which so often go hand in hand with the destruction of habitats. The dynamics of this trend is not yet sufficiently known. There are already many cases known where virulent new weeds invade ecosystems. Insular ecosystems are especially fragile and need to get much more attention regarding introduced new genomes in future. Examples are Guava on Mauritius and *Pittosporum undulatum* or *Goldfussia (Acanthaceae)* in Jamaica. We remind here also to cases like the „golden death“ *Senecio canadensis* and related species, *Reynoutria japonica*, *Lactuca serriola* and many other species which are beginning to threat certain ecosystems in peninsular Europe.

One has to bear in mind that the above mentioned rules are derived from the *„exotic species model“* and therefore a close comparison has its pitfalls as is pointed out by Scholz (1993), one of the foremost experts in exotic plant species. On page 99 of the same TA cited above, he comments:

> All species chosen by Sukopp and Sukopp 1994 as examples of naturalised exotic species are wild species or cultivated wild species, not crops. Scholz does not see any possibility of a close comparison between crop and cultivated wild species. Crop plants, even in the phase of escaping their agrosystems, are not wild species and cultigeneous mutants behaving as wild species cannot be compared in their genotype and phenotype with truly wild species. Cultivated species close to their wild relatives have phenotypes and genotypes different from crops.

Examples of cultigeneous wild species, which are interpreted by Scholz as mutants are *Avena sativa subsp. fatua* (Scholz 1991) and *Panicum miliaceum subsp. ruderale* (Scholz 1983). Wild species having adapted to agrosystems successfully and thus becoming weeds are *Echinochloa crus-galli* in rice fields (Barrett 1993) for example. In all those cases it will be necessary to consider seriously the risks and the appropriateness and the consequences of transferring transgenes.

We should bare in mind the basic differences between gene flow caused by novel transgenic crops and invasive species. Whereas in transgenic crops a new gene flow risk stems from single genes, gene introgression by invasive plants is done by moving alleles in huge packages. This will not cause necessarily the same risk effects, since position effects may work differently (if at all) in both cases during hybridization. Some transgenes will be so novel that the risk of their introduction in ecosystems is virtually unknown. Some transgenes will be comparable to genes introduced into crops by classic methods in their effect on ecosystems.

According to the situation in Great Britain Williamson (1988) estimates that most of the future transgenic plants could escape occasionally their agrosystems and some 10% will be able to establish themselves in some niches, and again 10% of those could become problems in their new ecosystems. Unfortunately, Williamson does not indicate which species and crops he bears in mind, but anyway this would be impossible at present time.

What can we learn from this discussion? A scientifically sound risk assessment has to follow a *case to case strategy*, considering the *reproductive and synecological properties, the biogeographical situation* and also the *nature of the transgene.*

4.3. Is there any difference between transgenic and non-transgenic plants ?

4.3.1. Are there genes for weediness existing ?

This question cannot be answered clearly. Such a lacuna in knowledge is also stated by Gray (1986). There are no clear-cut definitions of what a weediness gene should look like. There are no consistent characters of weediness anyway, since there are a multitude of genes responsible for weediness in complex ecosystems. For more details see also 3.8. in chapter weediness.

4.3.2. The case of *Brassica napus,* oilseed rape

A widespread crop which still is very close to its wild relatives is e.g. *Brassica napus,* oilseed rape:
Crawley et al. (1993) were unable to show significant differences between non-transformed and transgenic oilseed (kanamycin resistance and glufosinate tolerance) in their field experiments. They argued, that non-transgenic rape plants are slightly outcompeting their transgenic crops. This result shows that there will not be any obvious and short term invasion possible in case of an escaped gene, as long as the transgenes do not cause a major change in the ecological behaviour of the novel oilseed rape. Still, the ultimate proof that transgenic oilseed rape is harmless compared to its non-transgenic relatives is not yet delivered, although Crawley's experiments are quite laborious and ingeniously planned: 12 different habitats where chosen to test a mixture of transgenic and non-transgenic oilseed rape. It has been criticised repeatedly that the discussion of the results should not be based on mean values solely, since we must also take into account founder populations with unusual ecological behaviour, which could make those plants more vigorous than others, cf. Weber (1995), see also Sukopp and Sukopp (1993).

Poulsen et al. (1995) argues that differences in yield between cultivars are often very small, and in order to get significant results, cultivar testing normally requires larger numbers of replicates and larger plot sizes than used there.

In field experiments assessing competitive ability of transgenic oilseed rape (Fredshavn et al. 1995, Poulsen et al. 1995), the competitiveness of two transgenic oilseed rape lines and their fertile transgenic hybrid was tested in field trials in Belgium and Denmark. The lines contained genes for male sterility, restoration of fertility, kanamycin resistance and tolerance to the herbicide glufosinate. The competitiveness of these three transgenic lines was related to three non-transformed commercially-grown oilseed rape varieties. As a reference to a more aggressive crucifer, white mustard (*Sinapis alba*) was used in the experiments. All crucifers were grown in monocultures and mixtures with barley (*Hordeum vulgare*). The results show that the transgenic lines behaved similarly to the non-transformed cultivars. Despite the different growth conditions in Denmark and Belgium, the monoculture yield on the two locations is not remarkably different. In their field experiments, the authors observed a change in growth behaviour towards that of the reference weed *Sinapis alba* which could be an indication of increased weediness of one of the transgenic oilseed rape lines. For Poulsen et al. (1995), the exact consequences of a release are impossible to predict. To detect any basic change in growth behaviour, she proposes to test the transgenic plants in critical phases of the life cycle and to compare it with a range of nontransformed well-known cultivars.

It may be appropriate in cases of novel transgenes to apply a method to assess competitiveness proposed by Fredshavn and Poulsen (1993). It allows a scientifically sound comparison between transgenic and non-transgenic crops with regard to their competitiveness. According to the authors even this refined experiments cannot solve all questions totally. Only a field release in reality will take into account most important factors determining competitiveness. The last open questions will anyway be solved in the course of mass releases over many years to come. This is a harsh but realistic statement, which has become true in many cases of classical breeding as well. See also the discussion on *Brassica* in chapter 3.10. and the following chapter 4.3.2.

4.3.3. Field experiments with transgenic crops, a summary

In the light of the above somehow contradictory statements, generalisations would be premature. The situation calls for a case to case strategy. In various review papers impacts of gene transfer have been evaluated (Ahl Goy et al. 1994, 1995 and 1996).

However, there is a possibility to produce more clear statements in agrosystems with only a few decisive factors influencing the ecosystem: Clearly, a competitive advantage is produced, when wild relatives receive a herbicide tolerance gene from the transgene cultivar. But again one has to stress that this advantage can only be effective in a situation with continuous application of the pertinent herbicide. This has been shown in a parallel case (without transgenic plants being involved) by Gressel et al. (1983).

Despite these uncertainties it is possible to leave the grey zone of pro-active and contra-active guesses, which so often rule the discussion about genetic engineering:

Working through some 73 field release experiments (Crawley 1992, Crawley et al. 1993 and Fredshavn and Poulsen 1993), these authors did not reveal any hints that any kind of transgene would enhance competitiveness and therefore cause invasions of crop plants into natural habitats.
It has once more to be clearly stated that there are no cases known where a transgene has caused a considerable change in the ecological behaviour of the plant.
These studies cited above are based on the following transgenes: Phosphinothricine-resistance (Basta ® - resistance in oilseed rape and maize), the glyphosate-tolerance (Roundup ® - tolerance in sugar beet) and the insect-resistance (through bacillus thuringiensis-δ-endotoxine and lectine in potato). In most cases transgenic crops received genes such as Kanamycin tolerance or a GUS gene serving as a marker gene. There have been included the following characters of transgenic crops:
germination, dormancy, embryo and seedling resistance, growth, pest frequency, morphogenesis of the flower, fruit ripening, seed production and seed weight.

In oilseed rape, maize, potatoes and sugar beet there has been no stated enhancement of weediness through transgenes. In a model case Gressel et al. (1993) demonstrated that non-transgenic herbicide tolerance in *Brachypodium distachyon* can be favourable under conditions assuring selective pressure through herbicide application. In case of cessation in the application this tolerant variety of wild relative will vanish rapidly (see chapter 3.9.1.). But again there is no premature generalisation possible. For more details see also OECD (1993a) and Wrubel et al. (1992).

Williamson (1993) recognises that an efficient monitoring system is necessary to detect transgenic crops turning into pests. He showed at the example of *Impatiens* in Britain (see chapter 3.2.2.) that for the prediction of pest status, the variation in population behaviour in different habitats and the perceived value of those habitats must be considered. From the results of his detailed study of *Impatiens* and his survey of the weeds in the flora of Britain he concludes (p. 223):

> Pest plants differ from other closely related plants only in a small number of characters. The important differences ecologically are difficult to quantify. The important characters vary from case to case. The implication for the regulation of the release of GMOs is that minor gene differences can have major effects. This alone justifies proper field trials and monitoring.

Williamson (1993) thinks that some of the novel life forms of GMOs, not necessarily those that appear to have novel ecological characters, will become invasive. He estimates that the proportion of these novel GMOs that will become invasive or otherwise undesirable is small.

> But the history of invasions and other environmental disbenefits shows that the costs of those that misbehave is large. This is a familiar situation in insurance, and the same principle applies to the regulation of GMOs. A small recurrent cost is justified by the protection it gives against a large and unpredictable loss.

Generally, Crawley (1992) warns of unforeseen rare events (p. 50):

> Finally, Murphy's (Safety of Genetic Releases) Law should be borne in mind. This states that the biggest problems with genetically engineered organisms will come from those that look to be the 'safest' (i.e. from those cases where we perceive no risk at all). This is because, in cases where the organisms were perceived to be dangerous from the outset, such great care would be taken that problems would be spotted early, and eradicated. The unforeseen rare event, that creates a novel kind of problem from a benign-looking crop, will be difficult to control because, once released, it is not practical to eliminate plants from all the habitats, and from all the seed banks, into which they have found their way.

4.4. The risk of vertical gene flow caused by transgenic crops in Switzerland

4.4.1. General remarks

Possible negative effects of vertical and horizontal gene transfer
Up to now, no negative effects have been reported, originating from vertical or horizontal gene flow of transgenic crop plants released in the field.

Factual ***horizontal gene flow*** of transgenes into natural or semi-natural habitats has not been detected until this date. All proven cases of horizontal gene flow have been provoked in artificial systems or small scale field experiments, where a high gene flow pressure by means of favourable environmental conditions has been provoked. Horizontal gene flow between higher plants to soil microbes or aphids thus remains hypothetical, despite some papers cited in Altmann et al. (1992) (ref.18-20). Consequently, negative effects based on horizontal gene flow are hypothetical as well. (Schlüter et al. (1996), see both papers for more literature).

Principally, transgenes can be transferred through ***vertical gene flow*** to other species by transmitting pollen to compatible species or varieties.

Through vertical gene flow some unwelcome characters could be transferred from crops to wild relatives. Gene introgression, a well known phenomenon in population genetics since Anderson and Hubricht (1938), is by no means something new and is not connected solely to transgenic plants. In relation to gene flow of transgenes there is a true flood of smaller and more extensive review papers produced (Abbott 1994a,1994b, Ammann 1995, Bazin et al. 1995, Bevan et al. 1995, Ellstrand 1992, Gregorius et al. 1993, Raybould et al. 1993, 1994, Snow et al. 1995, Tiedje et al. 1989, van der Meer 1993). Lots of ingenious pollen release experiments have been carried through, over 1600 field trials have been counted until 1994 according to extracts from GIBiP Database on field trials 1986 - 1994 (Green Industry Biotechnology Platform 1995).

Still, there is no evidence for a clearly negative event due to vertical gene flow known. But it also has to be stated, as Wrubel et al. did in 1992, that many of those field releases have not been properly monitored, most of them lack a long term perspective, some even introduce report statements without data. There is no reason to underestimate the future risks of field releases of transgenic crops, since possibilities can be seen in rather realistic scenarios with oilseed rape and oat grasses (Ammann 1995). Nevertheless, we should not generate unnecessary anxieties. There are many crop plants where there is no or only minimal risk of vertical gene flow seen in future. Examples are: barley *(Hordeum vulgare)*, rye *(Secale cereale)*, maize *(Zea mays)*, potato *(Solanum tuberosum)*, tomato *(Solanum esculentum)*, soy bean *(Glycine max)*.

Tomiuk et al. (1993) discuss with care establishment and persistence of transgenic populations. There are a multitude of factors involved in the judgement of presumable risks. Distribution and colonising rates, frequency, reproduction modes, effects of human activity are among the most important. Biogeographical aspects come in where there are overlapping areas to be considered. Last but not least, sheer coincidental events can also influence the processes. It is not easy to generalise factors which can enter models in a simple way. All models considered in the paper cited here are built on rather simple scenarios and cannot be taken for granted, rather they can lead to a more disciplined argumentation about the risks in complex ecosystems. Changes in growth rate, influenced by environmental factors will have to be considered also. Even badly adapted populations of weak individuals can persist as transgenics in such models for a long time, since they can outplay their competitors by other favourable characteristics.

4.4.2. Escaped transgenes

Although it is true, that escaped genes are basically not retrievable, there are in the view of population genetics many things to be added to this oversimplified statement:

Through outcompeting and backcrossing there are considerable chances that an escaped gene will not persist in nature beyond several decades. Also it may not be expressed as a transferred gene. There are possibilities that escaped genes disappear again, there is no guarantee that transgenes survive in nature forever. We can assume that escaped transgenes survive in nature for long periods only under special circumstances. Risk assessment scenarios should nevertheless be based on the assumption that transgenes can survive in nature and may eventually do some considerable harm under specific conditions, since ecosystems are by far too complex to predict precisely what will happen.

4.4.3. What happens if a transgene escapes and a transgene population of a wild relative persists in nature for a long time ?

These questions have been answered already in the previous paragraphs. Processes, which lead to notable changes in the structure of biodiversity of a habitat, will not be predictable precisely. They may, in a worst case scenario, lead to the establishment of a new weed, which will persist in agrosystems. Competition in natural habitats still undisturbed will be strong enough to keep these new weeds out. In the table (see 4.6.1.) we will have a look at the most important crop plants and cultivars in order to find out about potential artificial vertical gene flow. The nature of the transgene and possible position effects may have an influence on the dynamics of the processes.

This artificial gene flow is already happening now without transgenes and has started the moment domestication began.

4.4.4. Some selected case histories

4.4.4.1. Transgene spread from oilseed rape

In the case of oilseed rape there has been recently a publication by Mikkelsen et al. (1996) showing evidence of transgene spread. The results suggest a rapid spread of transgenes through interspecific backcrossing under field conditions. The occurrence of fertile, transgenic weed-like plants after just two generations of hybridization should be taken into account when considering the consequences of transferring new traits to oilseed rape. The wild species mentioned in this article is *Brassica rapa ssp. campestris* (often referred to as *B.campestris*). It seems that this species (according to Danish and Swiss experience) coexists with *Brassica napus*, oilseed rape. It will hardly be able to occur outside the range of B. napus. Consequently, the authors themselves give a rather pragmatic interpretation of their own results: It depends strictly on the nature of the transgene whether there will be a rapid spread of the new transgenic weed or not. In the case of the transgenic weed obtained by their field experiments they cannot see any dramatic effects in the future.

This is also the opinion of Timmons et al. (1996): The research group of the Scottish Crop Research Institute found in their own study area of Angus, Scottland, intercultivar hybrids at a distance of 360m of a transgenic oilseed rape field. They conclude that attention should now focus on the probable effects of any introduced transgenes. The group infers, that in the area surveyed, the possession of glufosinate-tolerance is unlikely to affect the survival of feral populations significantly, since at present time there is no extensive use of glufosinate in non-agricultural situations. This assertion takes, according to the authors, no account of future changes in glufosinate application in non-agricultural situations, of pleiotropic effects of the transgene, or of effects on agricultural volunteers. Their work demonstrates once more the need for a careful, case-by-case approach to the risk assessment of genetically modified organisms.

4.4.4.2. Possible transgene spread from oat

Although there have been specific warnings, Somers et al. (1992) transferred without hesitation herbicide resistance into cultivated oat, a wind pollinated cultivar highly compatible with its wild relatives, among those some bad weeds such as *Avena fatua*. See also Bryant (1992), Boudry et al. (1993). Warnings came early enough to prevent field releases.

4.4.4.3. Possible transgene spread from sugar beet

Also in the case of sugar beet there are scenarios visible, which should be taken into account in the risk assessment.

Sugar beet has been modified with transgenes already with herbicide tolerance against BASTA, Boudry et al. (1993). There is now a real possibility that the transgene can escape through hybridization with hybrid beet, see 3.7.2. Without any genetic engineering influence there has been produced a new weedy beet in north-western Europe. *Beta vulgaris subsp. maritima* has hybridised with *Beta vulgaris* and now is becoming a noxious weed in beet agrosystems, since the weedy beet is germinating and flowering one to two months earlier than the cultivar, the hybrids thus behave like annuals. These weedy beets have now some selective advantage against the crops. If they get herbicide tolerance through vertical gene flow (hybridization), it will be even more difficult to fight this new pest. Dale (1992), Graner et al. (1993) and also the authors, consider in a case of future field releases, which must be carefully evaluated, measures should be taken to organise biological containment in order to avoid undesirable escape of transgenes.

4.4.5. Summing up the risk of escaped transgenes

In the course of this risk assessment study we have been confronted with a huge amount of new pertinent publications. Many of these publications also reflect a new awareness regarding risks not only with upcoming genetic engineering but also with classical breeding.

Certainly we also have to take into account the *„human factor“*, which may, combined with a certain amount of sheer ignorance, cause considerable difficulties. If we look at these risks from the perspective of gene introgressions, we have to realise, that we have lived for a long time already in this situation. A concrete example of how the human factor could work, is the case cited above of transgenic *Avena sativa*.

Trying to persuade the public that there are no risks to be considered, is wrong already. Although there is only one concrete example of an escaped transgene (in oilseed rape, see above), there might be several other *possibilities* of introducing harmful transgenes to nature, which have not been realized up to now. Still, one has to state that truly negative *effects* on ecosystems are not known up to now. What we need are strict regulations, which prevent negative scenarios of becoming realities in most cases. But there is no way to achieve absolute safety, as it has always been with the introduction of new technologies.

For most people working with transgenic crop it is beyond any doubt that it is highly important to include thoughts about the socio-agraric system influenced by such activity (Leisinger 1991, Theisen 1991, Altmann et al. 1992, Keeler et al. 1991).

4.5. Risk of insects becoming immune to the biopesticide Bt

It will be necessary to adopt more careful use of the biopesticide Bt (a gene coding for a protein turning toxic in the intestines of certain pest insects).
More than 29 Bt genes have been sequenced (Feitelson et al. 1992) and some genes have been artificially modified to enhance protection against recalcitrant Lepidoptera (van der Salm et al. 1994).
It has been shown by Whalon (cited in Anderson 1992) that Colorado beetles bred in the laboratory show a 200-fold increase in Bt-resistance in only 17 generations. The same author claims that a comparable evolution would take just five years in nature. Overuse of Bt biopesticide spraying in organic farming has already produced some insects twice as resistant as those just a few generations ago. There are several well documented cases of diamond back moths having aquired resistance following traditional bt application by spraying (McGaughey 1985, Ferre et al. 1991, Tabashnik et al. 1991, Williams et al. 1992, Tabashnik 1994).
Broad spectrum resistance in *Heliotis virescens* (cotton-bollworm) has been obtained in laboratory conditions after 17 generations, where resistance ratio climbed from 2 to 10 in 10 generations, and then from 10 to 20 in 16 generations. Finally, with the 17th generation there was a dramatic increase to 50 noted (Gould F. et al. 1992, see also papers of the same author on integrated pest management and evolutionary biology and genetically engineered crop from 1988a and b).
Gould et al. op.cit. make several proposals how to deal with the resistance problems. They conclude that genetically engineered plants could help limit the use of environmentally disruptive synthetic chemicals. As such, pest-resistance genes are an important natural resource. But if these genes are widely used in a homogeneous commercial crops, pests may adapt to them and cause rapid loss of this useful natural resource. More sophisticated approaches for engineering resistant plants are becoming available and could lead to the production of more stable resistant crops. These new approaches will, however, require greater intellectual and capital investment. According to Snow et al. (1995) it has still to be clarified properly, which insects within the broad categories such as lepidoptera are susceptible to which bt toxines.
Certainly, we can learn a great deal from classical breeding, where it has been demonstrated for a long time, that resistance based on several genes is more stable in new cultivars than resistance based solely on one gene. (Incidentally: who cared about field releases of these newly resistant cultivars at the given time of their introduction to mass production? Gene flow could well have been a problem then too!). For more information about managing newly acquired resistance genes see McGaughey et al. 1992, Mallet et al. 1992 and van der Salm et al. 1994.

The application of Atracine in Switzerland in an extensive way has caused more than 10 weeds resistant to Atracine (it should be noted that this happened without genetically engineered plants, but simply by an application of a herbicide which is much too efficient in performance). According to Ammon et al. (1990) some *Sorghum*-species and maize can decompose Atracine to inefficient components. In addition, chloroplastic resistance has been developped in *Chenopodium album L.* and later also in *Amaranthus retroflexus L.* and several other weeds.

4.6. Risk assessment in Switzerland regarding transgenic crops

A scientifically sound risk assessment requires a multidisciplinary approach and even more so a truly interdisciplinary procedure to filter out solutions acceptable for everyone. Therefore we need a considerable amount of scientific research which is oriented towards problem solution on practical grounds. (Altmann et al. 1992, Ammann 1994).

We should continue risk assessment research following the lines given below:

4.6.1. List of crops and cultivars of some importance in Switzerland, which could be subject to genetic engineering in future

We have put together a list of crop and cultivar plants growing in Switzerland and their potential hybridising wild relatives in Europe (derived from Ammann et al. 1994 and Heywood and Zohary 1996), the latter being a new list which comprises the surprising number of 397 taxa (including all subspecies and some varieties mentioned, including all medicinal plants, kitchen herbs, aromatic herbs, forest species and ornamental plants, which are left out here).

The list given here is inevitably incomplete, it contains only crops and cultivars which are cultivated in Switzerland to an important extent. In the right column there are all wild or feral plant species and subspecies in Europe listed, which can produce hybrids with these crops. In the left column only crops and cultivars are mentioned, which may be of some importance in Switzerland.

Cultivated Plants	**Wild Relatives**
Here only the most important crops for the Swiss perspective are given:	Here all European species and subspecies are given, which may be subject to vertical gene flow:
Beta vulgaris L. subsp. rapacea sugar beet, red beet, fodder beet	**Beta vulgaris L. subsp. maritima (L.) Arc.** Wild beed native to the coasts of W. and S.Europe
Brassica napus rape, swede	**Brassica oleracea diploid and B. rapa L. ssp. campestris (L.) Clapham diploid** Wild rapes and wild cabbages of Europe
Brassica oleracea L. cabbage, cauliflower, kohlrabi, Brussels sprouts etc.	**Brassica oleracea subsp. oleracea s.str., B. montana Pourret, B. rupestris Rafin, B. villosa Biv. B. incana Ten. B. macrocarpa Guss., B. cretica Lam., B. hilarionis Post**
Brassica rapa L. turnip	**Brassica rapa L. subsp. campestris (L.) Clapham diploid** Weedy and ruderal forms of turnip
Raphanus sativus L. radish	**Raphanus raphanistrum L.** wild radish
Apium graveolens L. celery, celeriac	**Apium graveolens L.** wild forms of celery
Carum carvi L. caraway	**Carum carvi L.** wild forms of caraway
Daucus carota subsp. sativus & subsp. carrot	**Daucus carota subsp. carota, subsp. gummifer Hooker fil., subsp.commutatus (Paol.) Thell., subsp. hispidus (Arcangeli) Heywood, subsp. hispanicus (Goaun) Thell., subsp. gedecaei (Rouy et Camus) Heywood, subsp. drepadensis (Arcangeli) Heywood, subsp. rupestris (Guss.) Heywood** wild carrots
Foeniculum vulgare Miller fennel	**Foeniculum vulgare Miller var. piperitum (Ucria) Coutinho** wild forms of fennel
Pastinaca sativa L. parsnip	**Pastinaca sativa L.** wild forms of parsnip, very variable
Petroselinum crispum (Miller) A.W.Hill parsley	**Petroselinum crispum (Miller) A.W.Hill** wild forms of parsley
Malus pumila Miller (=M. domestica Borkh.) apple	**Malus sylvestris (L.) Miller subsp. sylvestris, subsp. orientalis (Uglitzkich) Browicz (=M. orientalis Uglitzkich)** wild crab apple
Prunus avium L. sweet cherry	**Prunus avium L.** wild forms of sweet cherry
Prunus domestica L. plum	**Prunus cerasifera Ehrh.** cherry plum
Pyrus communis L.	**Pyrus pyraster Borkh. with P. pyraster Borkh. subsp. caucasica Fedorov, P. elaegnifolia Pallas, P. spinosa Forssk. (= P. amygdaliformis Vill.), P. nivalis Jacq.**

pear	wild pear and subspecies
Lupinus angustifolius L. blue or narrow-leaved lupin	**Lupinus angustifolius L.** wild or naturalized forms of blue lupin
Medicago sativa L. subsp. sativa alfalfa, lucerne	**Medicago sativa L. subsp. falcata (L.) Arcangeli, subsp. glomerata (Balbis) Tutin, subsp. coerulea (Less. ex Ledeb.) Schmalh.** wild lucerne subspecies
Phaseolus vulgaris L. bean	**Phaseolus vulgaris L., Phaseolus coccineus L.** feral old varieties of bean, Scarlet bean
Pisum sativum L. **vum** garden and field peas	**Pisum arvense L.** (often included in Pisum sativum), **Pisum sati-** **L. var. elatius (Bieb.) Ascherson & Graebner** wild peas
Trifolium repens L. cultivars of white clover	**Trifolium repens L. subsp. repens** wild varieties of white clover
Trifolium pratense L. cultivars of red clover	**Trifolium pratense L.** wild red clover and maybe **T.medium, T.alpestre, T.rubens**.
Vicia sativa L. **(Dor.)** **&** **sativa,** common vetch	**Vicia sativa L., subsp. nigra (L.) Ehrh., subsp. amphicarpa** **Aschers. & Graebn., subsp. cordata (Wulfen) Aschers.** **Graebn., subsp. incisa (Bieb.) Arcangeli, subsp.** **subsp. macrocarpa (Moris) Arcangeli** wild forms of common vetch, very variable
Linum usitatissimum L. flax, lineseed	**Linum bienne Miller** biennial flax
Cichorium endivia L. var. sativum Lam. & DC. endive (anglice), escarole **Cichorium intybus var. foliosum Hegi** witloof	**Cichorium endivia L.** wild forms of endive
Lactuca sativa L. lettuce	**Lactuca serriola, L. virosa L., L. saligna L.** wild lettuce, prickly lettuce, willow lettuce
Allium cepa L. onion	**Allium cepa L. , Allium fistulosum** feral populations of onion, winter onion
Allium porrum L. leek	**Allium ampeloprasum L.** wild garlic
Allium schoenoprasum L. chives	**Allium schoenoprasum L.** wild forms of chives
Asparagus officinalis L. asparagus	**Asparagus officinalis L.** wild asparagus
Avena sativa L. oat	**Avena byzantina C.Koch** (often included in A. sativa), **Avena sterilis L., A. fatua L.**
Dactylis glomerata L. cock's foot, orchard grass	**Dactylis glomerata L. subsp. aschersoniana (Graebn.) Thell., subsp. lusitanica Stebbins & Zohary, subsp. juncinella (Bory) Stebbins & Zohary, subsp. ibizensis Stebbins & Zohary, subsp. reichenbachii Hausm. ex Dalla Torre & Sarnth., subsp. smithii (Link) Stebbins** wild tetraploid forms and several diploid subspecies
Festuca pratensis Hudson fescue	**Festuca pratensis Hudson, var. apenina (De Notaris) Hegi** wild fescue
Festuca rubra L. red fescue, cultivated forms	**Festuca rubra L.** red fescue, many wild subsp. and varieties
Hordeum vulgare L. barley	**Hordeum spontaneum C.Koch, H. nodosum L.** wild barley, progenitors of H.vulgare, knotty barley
Lolium perenne L. cultivars of perennial ryegrass	**Lolium perenne L.** wild forms widespread
Lolium multiflorum Lam.	**Lolium multiflorum Lam., Lolium temulentum L.**

cultivars of Italian ryegrass	wild forms of Italian ryegrass and bearded darnel
Phleum pratense L. timothy	**Phleum pratense L.** wild timothy, morphologically not distinguishable from cultivated traits
Secale cereale L. rye	**Secale cereale L., Secale montanum Guss.** wild and weedy forms of rye
Setaria italica subsp. italica foxtail, Italian millet.	**Setaria italica subsp. viridis** green foxtail
Triticum aestivum L. cultivars of wheat	**Triticum aestivum L.** old cultivars, **T. turgidum L, T. boeoticum Boiss. T. speltoides Tausch** old cultivars, also hybridizes artificially with **Secale cereale**
Zea mays L. maize	**Zea mays L.** old cultivars of maize

The list demonstrates, that hybridization has been possible already in the times of classical breeding and there are many known cases of dynamic displacement through competition. The phenomenon of vertical gene flow has a long tradition in classical breeding.
For Switzerland the subspecies or species in the right column can be considerably reduced.

One case is particularly well studied: *Medicago sativa* subsp. *falcata* is under heavy pressure from *Medicago sativa* subsp. *sativa* in present times. It seems that *Medicago sativa* subsp. *falcata* has become a threatened species in many regions of Switzerland: It is to-day very difficult to confirm many locations which are still documented in the Swiss flora atlas from 1972 (Welten and Sutter 1972). *Medicago sativa* L. subsp. *falcata* is driven out not only for reason of habitat loss (mainly through heavy nitrogen input) but also through population pressure. This pressure has increased through the massive cultivation of aggressive traits of *Medicago sativa* L. which have been introduced from abroad.

Risk assessment strategies should take into account these facts. This does not mean that here we try to construct inferior excuses for future vertical gene flow provoked by novel genes. On the contrary: it will sharpen our mind. We should try to learn from these events and also extend our risk assessment discussion to classical breeding as well. Again we have to stress that focusing on genetic engineering is wrong. In consequence of this we also should extend legislation on field releases to certain cases of classical breeding.

But the argument remains valid that also for classical breeding we can postulate negative impact on ecosystems through vertical gene flow to wild species, but this has not a been a contentious topic until now. There are no tests known to the authors and certainly no opposition from ecologists. For many decades of intensive and effective classical breeding, which resulted in hundreds of genes (actually alleles!) put into crops, which undoubtedly also introduced into the wild, totally new gene flows. There are no known negative events up to now.

4.6.2. Conclusions

What we actually need in Switzerland (and most probably elsewhere) is a research centre staffed with at least some 20 highly skilled scientists from all disciplines, from pure science in genetic engineering to pertinent social sciences and philosophy of sciences. This research centre should be funded by governmental institutions. There is no doubt that this new and important technology deserves more appropriate and permanent risk assessment and related research. This new research institute should not be run in reverse gear but take on the new tasks, which means taking a new and critical look at monocultures, often the source of negative effects on ecosystems. The activity should start with an inventive attitude tending to introduce ecological thought in new breeding strategies, also it should distinguish between potential risk and truly negative effects. In addition this institution should make it clear that long term monitoring is an essential part of risk assessment. Consequently, one of the prior-

ity tasks would be to develop a concept for risk assessment embracing short term strategies in confinements up to long term monitoring of mass field releases for commercial purposes.
We should learn from history, that introducing new technologies always goes parallel to major changes in the world of labour: It would have been senseless to stop the development of railway systems with the argument of the vanishing horse-drawn vehicles. Modern (classical) breeding of sugar beet has caused major damage to the sugar cane industry of the tropics, to give just one example. We should learn from such negative events and try to introduce genetic engineering in view of these negative after-effects. There is no such rule that genetic engineering must necessarily lead to negative effects.
Also it should be stated explicitly, that risk assessment discussion should not focus alone on genetic engineering, since the risk of negative effects of gene flow on ecosystems cannot be restricted to transgenic crop. Genetic engineering should be part of the methodology of classical breeding and with a new and critical view on some developments in classical breeding.

4.6.2.1. Conclusions regarding agricultural strategies

It should not be regarded as the ultimate and only solution to produce new (or novel) transgenic crops when it comes to evaluate progress in breeding.

We should have a new look at cultivation methods which avoid some of the major drawbacks of monocultures, which often are the reason for pest problems. Genetic engineering is a new breeding technique which could help greatly in shifting towards an ecologically more sound agriculture. In future there should be a perspective of organic farming adapting the advantages of genetic engineering. This still needs a lot of rethinking about basics in breeding strategies in connection with ecologically sound agriculture. Biotechnology can be promising for sustainable agriculture, but it needs to adapt to its needs. As long as the economic need for sustainability has not been generally accepted, there is little hope of change to a trend, where genetic engineering and organic farming can effectively communicate and develop combined strategies (Barret 1992).

4.6.2.2. Conclusions regarding field releases and breeding strategies

As Dale (1992) points out, there are many factors involved determining a truly viable hybrid:

Production of viable hybrid seeds

1. Compatibility of the two parental genomes (mitotic and genetic stability)
2. Ability of the endosperm to support hybrid embryo development
3. Direction of the cross: one parent may support embryo and seed development than the other.
4. Number and viability of hybrid seeds

Establishment of hybrid plants from seeds in soil

5. Seed dormancy
6. Vigour of the hybrid plant
7. Direction of the cross: maternal effects influencing seedling vigour
8. Nature of habitat: wild, semi-wild or agricultural
9. Nature of competition from other plants
10. Influence of pest, diseases and animal predators

Ability of the hybrid to propagate vegetatively and sexually

11. Method of vegetative propagation
12. Persistence of vegetative propagules in agricultural habitats
13. Dissemination of vegetative propagules
14. Invasiveness of vegetative propagules in natural habitats
15. Sexual breeding system: cross compatible, self compatible, ability to cross to either parental species
16. Male and female fertility: meiotic stability and chromosome pairing
17. Seed number and viability
18. Seed dormancy
19. Nature of habitat: wild, semi-wild or agricultural
20. Nature of competition from other plants
21. Influence of pest, disease and animal predators

Any risk assessment should deal with (1) transgenes, (2) its species or crop trait and its biology involved and also (3) its biogeography. This is also confirmed by a report of Jacot et al.

(1994). Generalisations are only possible where one finds clear-cut parallel cases. Biogeography can limit generalisation considerably. One example is: in Europe and Asia vertical gene flow from maize to its wild relatives can be ruled out (except for a gene flow to ancestral maize cultivars), but this is not the case in Central America, where maize has its natural wild relatives. Even in Central America this might not be of great importance when considering certain harmless transgenes, however, it might well not be judged as being harmless in other cases.

In a comprehensive risk assessment on crops bearing the transgene Bt has been organised by German authorities and reviewed by Altmann (1994). Sukopp and Sukopp (1994) included some interesting comments on the *exotic species model*. They propose 5 measures to be taken in order to minimise gene flow:

- GMO's should have a maximum agricultural specificity. This means that GMO's will be bound to agricultural systems in an optimal way.
- Predictability must be possible, although it is known from experience with invasive species, that there is always the possibility of unpredictable events.
- It must be possible to take back escaped transgenes. At any time a total destruction of transgenic populations must be possible.
- The reproductive strategy of GMO's must be known. Sterile or strictly self-fertilised crops which lack vegetative propagation for more than one vegetation period should be chosen.
- The chorology of a given cultivar must be known. If the GMO produces diaspores, the capability to spread must be restricted sincerely.

It would be ideal to apply all the above rules, but unfortunately they do not work in all cases as has been the case in classical breeding.

4.6.2.3. Conclusions regarding combined codes for the judgement of risk regarding field release of transgenic crops: Introduction to gene flow indices

As a result of discussions in the symposium at Louverain, we propose **gene flow indices** after the idea of some Dutch authors ([Frietema] De Vries et al. 1992, Frietema De Vries 1996) We are giving here an adapted version in order to spur discussion on an European level. We think it is desirable to establish a European classification system as proposed by Frietema De Vries (1996), where some of our proposals have been adopted. It is not possible to arbitrate the crops and their wild relatives on one and the same level all over Europe: Classification work has to be done on a *regional* scale taking into account local environmental conditions, species and transgenes. This regional scale has been proposed by Frietema De Vries (1996), following the well known subdivisions of Meusel.
Critical comments for the proposals given here are welcome. Here we deal only with the three first codes, but we feel strongly the necessity of a fourth code for the future: We need to assess also the *risk of the inserted transgene itself*. For this *Dg code* we need experimental approaches on all levels from a strict containment over small scale field releases to the large scale releases over long periods. For the time being there remains only the possibility of a rough estimate of how transgenes will have side effects in the long run, some comments are built in provisionally in code Dp (vertical gene flow). The authors are well aware of the pragmatic view they take, which is blurring the logic of the three codes already defined.

These codes are presented here in order to open debate on feasibility and organisation of such codes for future risk assessment:. The codes can serve as a first routh estimate, before going into more detail for a risk assessment based on field monitoring and experimental approach, where judged necessary.

Dpdf-codes, adapted to Swiss and European needs as a whole

4.6.2.3.1. Dp: Hybridization and pollen dispersal index

4.6.2.3.2. Dd: Diaspore dispersal index,

4.6.2.3.3. Df: Distribution Frequency index at present times

4.6.2.3.4. Classification by combination of the three codes :

4.6.2.3.1. Classification of the codes of dispersal of pollen (Dp)

Dispersal of pollen and hybridization potential, including a differentiation of possible negative ecological effects of the inserted gene itself. Categories 0 (lowest risk) to 5 (highest risk) and U (unknown)

Category Dp 0:

No chance for hybridization because there are no wild relatives growing in Switzerland. No ecological effects when the cultivated plants come into flower.
Monitored field releases possible, no containment experiments and no field experiments necessary.

Category Dp 1:

No chance for hybridization with wild relatives because it is experimentally proven that wild species of the same genus in Switzerland are not compatible with the cultivated plant: (artificial pollination methods and/or embryo rescue are necessary to produce hybrids).
No ecological effects when cultivated plants come into flower. Monitored field releases possible without containment. However, experiments should be carried out, to test there are no negative effects on the host / predator system in case of transgenes introducing new resistance and/or competition effects.

Category Dp 2:

No chance for hybridization with wild relatives because there is no record of spontaneously formed hybrids of the cultivated plant with wild species of the same genus in Switzerland.
However, hybridization is possible under experimental conditions and progeny is fertile without any artificial help. Chances of gene flow by hybridization is small due to various outcrossing barriers (competition, biogeographical or ecological incompatibility), but under special local or artificial conditions in agricultural systems still to be considered as possible rare events.

a) In certain species groups there is a small chance of getting new transgenic hybrids, but no invasions are to be expected.
b) In other species groups there is a small chance of getting new transgenic weeds which tend to be aggressive and will possibly cause invasions under unfavourable conditions.

Category Dp 3:

Natural hybridization occurs only occasionally, backcrosses have not been observed up to now. Local situations have to be studied carefully in risk assessment of field experiments. Species to species, region by region and step by step approach required.

a) In certain species groups and under unfavourable circumstances gene flow by pollen transfer will occur, but new transgenic hybrids do not tend to be invasive.
b) In other species groups and under unfavourable circumstances gene flow by pollen transfer can influence ecosystems negatively: Local invasions of new transgenic weeds will occur.

Category Dp 4:

Chance for natural hybridization is medium; backcrosses have been observed, successful outcrossing occurs fairly often. Natural fertile hybrids are sometimes observed, small hybrid populations can be detected in nature. Species to species, region by region and step by step approach required.

a) Transgenic hybrids will have no ecological effects on the flora of the Switzerland, since the new hybrid is only capable to invade small ecological niches, and therefore does not demonstrate any disturbing invasiveness, since the inserted gene itself did not show negative ecological effects in long term monitoring experiments. Experiments should also be carried through proving that there are no negative effects on the host / predator system.
b) Transgenic hybrids will have ecological effects on the flora of the Switzerland, since the new weed is capable to invade ecological niches, and therefore is potentially demonstrating invasiveness. There may also be negative effects (e.g. more competitive, more allelopathic) caused by the inserted gene itself.

Category Dp 5:

Chance for natural hybridization is high; vertical gene flow occurs often, hybrids are fertile and backcross frequently. Hybrid populations are often found in nature. Species to species, region by region and step by step approach required.

a) Transgenic weeds will have no ecological effects on the flora of Switzerland, nevertheless the new weed is capable to invade important ecological niches and it will act as a new weed (which should by all means be avoided!), but the inserted gene itself does not show negative ecological effects.
b) Transgenic weeds will have negative ecological effects on the flora of Switzerland since it is capable of invading many ecological niches as a major new weed and/or since the inserted gene itself may have characters demonstrating negative ecological effects.

Category Dp U:

Data too scanty or lacking at all, no evaluation possible.

4.6.2.3.2. Classification of the codes for the dispersal of diaspores (Dd)

Category Dd 0:

No chance for dispersal of diaspores to the wild: Seeds are sterile or otherwise deficient, they have lost reproductive function. No ecological effects are expected from fruiting of the cultivated plants.

Category Dd 1:

Dd to the wild occurs only occasionally and under very favourable conditions, plants usually survive only for one season (advena), they are not adapted for survival in our climate. No ecological effects are to be expected regarding the Swiss ecosystem.

Category Dd 2:

Chance for dispersal of diaspores to the wild is small, but under favourable and exceptional conditions possible. Further research on population dynamics seems necessary. For risk assessment the standing of the plants in the Swiss ecosystem can be of importance.

Category Dd 3:

Chance for dispersal of diaspores (by spontaneous vegetative reproduction) is real; fruiting of the cultivated plant is essentially undesirable and will normally be suppressed by various methods. Further research on population dynamics is necessary. For risk assessment the standing of the plants in the Swiss ecosystem can be of importance.

Category Dd 4:

Chance for dispersal of diaspores to the wild real. Fruiting of the cultivated plant occurs normally during cultivation. Ecological effects can be expected from fruiting of the cultivated plant. For risk assessment the standing of the plants in the Swiss ecosystem will be of importance.

Category Dd 5:

Dispersal of diaspores to the wild will be the rule. Fruiting occurs very frequently and also extremely abundant. Ecological effects can be expected from fruiting of cultivated plant. For risk assessment the standing of the plants in the Swiss ecosystem will be of importance.

Category Dd U:

Data too scanty or lacking at all, no evaluation possible.

4.6.2.3.3. Classification of the codes for Df (frequency of distribution)

Category Df 0:

No plants of this species or of a wild relative, no feral populations found in nature; no ecological effects are expected from the introduction of the cultivated transgenic plant.

Category Df 1:

Plants of this species or of wild relatives are extremely rare in the wild and have their stable place in the Swiss ecosystem in specific associations. No feral populations are found in Switzerland. Chances for hybridising with the wild or feral populations are negligible. Locations to grow transgenic plants should be appropriately chosen in order to avoid hybridisation and any ecological effect.

Category Df 2:

Plants of this species or of wild relatives are rare, but occur sporadically, distribution difficult to predict and essentially uncontrollable. Feral populations may exist in certain regions. Chances for hybridising with wild populations are scanty but unpredictable. Ecological effects from the introduction of the cultivated plant may be expected, but in most cases on a local scale only. Locations to grow transgenic plants should be appropriately chosen in order to avoid hybridisation and any ecological effect.

Category Df 3:

Plants of this species or of wild relatives are not very common in the wild and have their stable place in Swiss ecosystem. Feral populations are known from Switzerland, but not frequent. Chances of hybridising with the wild populations exist but are small. Some ecological effect from the introduction of the cultivated plant may be expected under unfavourable conditions when cultivated plants and wild relatives are not sufficiently separated. Locations to grow transgenic plants should be carefully chosen in order to avoid hybridisation and any ecological effect.

Category Df 4:

Plants of this species and their wild relatives are not frequent but well distributed over the whole Swiss plateau, chances for hybridising with wild populations are considerable, but under very favourable conditions it can still be safely prevented. Feral populations are known and distributed over an important part of Switzerland. Locations to grow transgenic plants should be carefully chosen in order to avoid hybridisation and any ecological effect. Detailed biogeographical studies are necessary to reach this goal.

Category Df 5:

Plants of this species and their wild relatives are common and well distributed over the whole Swiss plateau, chances for hybridising with wild populations must be expected and cannot be prevented in field experiments. Feral populations are frequent and distributed over the whole Switzerland. In exceptional cases locations to grow transgenic plants can still be carefully chosen in order to avoid hybridisation and any ecological effect. Detailed biogeographical studies are necessary to reach this goal

Category Df U:

Data too scanty or lacking at all, no evaluation possible.

4.6.2.5 Summing up the codes in a table:

Dp: Hybridisation and pollen dispersal index

Dp0: No wild relatives in Switzerland
Dp1: No compatible wild relatives in Switzerland
Dp2: No records of spontaneous hybrids in Switzerland
Dp3: Occasional natural hybridisation, no backcrosses observed in Switzerland
Dp4: Natural hybridisation occur and hybrids are fertile and do backcross.
Dp5: Natural hybrisdisation occurs fairly often, hybrids are fertile and do backcross frequently.

Dd: diaspore dispersal index

Dd0: No chance for diaspore dispersal (seeds are sterile or deficient)
Dd1: Diaspore dispersal possible occasionally under very favourable and exceptional conditions
Dd2: Diaspore dispersal possible under favourable conditions
Dd3: Diaspore dispersal occurs, fruiting is usually undesirable and is normally suppressed by various methods.
Dd4: Diaspore dispersal is important, fruiting occurs normally during cultivation
Dd5: Diaspore dispersal is the rule, fruiting occurs very frequently and is very abundant

Df: Dispersal frequence

Df0: Wild relatives not known in the wild or as feral populations in Switzerland
Df1: Wild relatives extremely rare in the wild and do not occur as feral populations in Switzerland
Df2: Wild relatives very rare in the wild and/or they occur sporadically as feral populations in Switzerland
Df3: Wild relatives and/or their feral populations not very common in the wild in Switzerland.
Df4: Wild relatives and/or their feral populations not frequent in the wild but well distributed over the whole plateau in Switzerland
Df5: Wild relatives and/or their feral populations common in the wild and well distributed over the whole Swiss plateau in Switzerland

4.6.2.6 Classification by combination of the three codes :

The goal of this study was to develop a convenient classification of gene dispersal probability from transgenic crop to the wild flora, adapted for Switzerland. After an evaluation of the three single factors (see above, dispersal codes), the combination of these codes enables us to estimate impact on the wild flora. Five categories of risk probability have been developed:

After an evaluation of the three single factors, their combination enables us to estimate the impact of a transgenic species on the environment. Six categories of risk probability have been developed:

1 No effect ▭

- No related species or no compatible related species of the crop are known in Switzerland. Field releases of species belonging to this category are possible without any containment or short term monitoring.
- Certain transgenes have to be tested in medium term field experiments regarding their secondary effects on ecosystems: Sustainable resistance must be achieved. To reach this goal a long term monitoring is required.

2. Minimal effects ▨

- No records of spontaneous hybridization between the crop and the wild relatives are known in Switzerland. Field releases are possible after a thorough clarification of the biogeographical situation. Short term monitoring in confinements should be done prior to large scale field releases.
- Certain transgenes have to be tested in medium term field experiments regarding their secondary effects on ecosystems (pest and insect resistance genes).

3. Low but local effects ▨

- Gene flow occurs towards wild or feral species existing also outside agricultural environment and control. Release experiments should first be done in confinements and afterwards in small scale releases closely monitored.
- This statement is restricted to transgenes not causing enhanced competitiveness outside agricultural environment, such as herbicide tolerance. Any other transgenes should be carefully tested in confinements.

4. Substantial but local effects ■

- Gene flow is high and substantial, but still locally controllable.
- Field releases could be done within strict confinements. A case by case analysis including the potential effects of the transgene is required before any field releases are done.

- Long term monitoring of field releases under strict biological or geographical confinement conditions is necessary in order to study competitiveness of the transgenic crop. Risky transgenes have to be avoided.

5. Substantial and wide-spread effects

▤

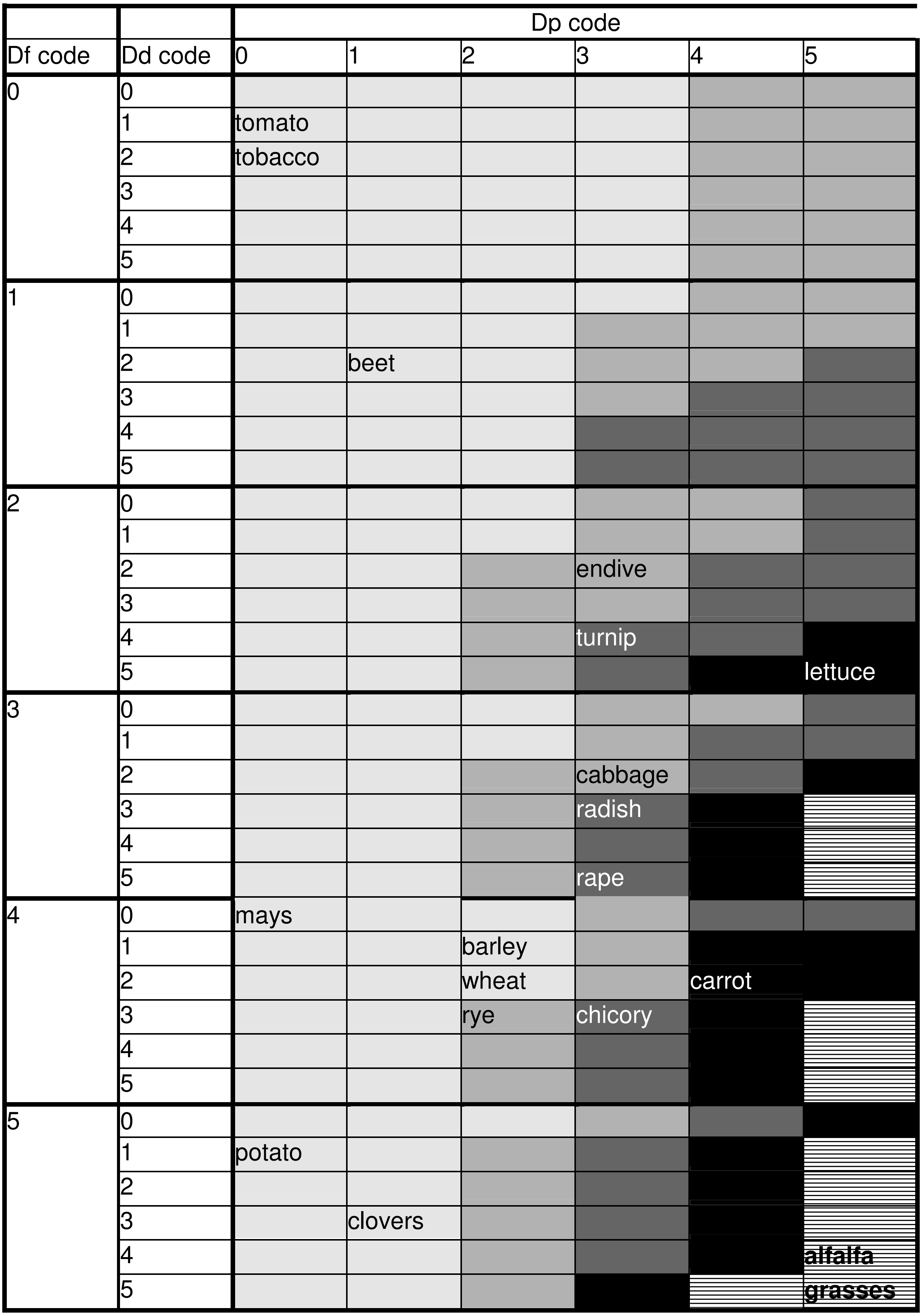
- Gene flow is high, substantial, and widespread and will not be controllable by any means.
- No field releases of species belonging to this fifth category are possible.
- Medium term monitoring under strict confinement conditions is necessary in order to find out about competitiveness of the transgenic varieties.
- Experiments with less risky crop varieties (e.g. with male sterility) having the same favourable effect desired.

Unknown (one of the three indices is unknown)
More studies are needed before any field releases are done.

4.6.2.7 Dispersal indices for some important Swiss crops and risk categories

species	English name	dispersal index Df.Dd.Dp	risk category
Festuca arundinacea	Fescue	5.5.5	Substantial and widespread
Festuca pratensis	Meadow fescue	5.5.5	Substantial and widespread
Lolium multiflorum	Italian ryegrass	5.5.5	Substantial and widespread
Lolium perenne	Perennial ryegrass	5.5.5	Substantial and widespread
Medicago sativa	Alfalfa	5.4.5	Substantial and widespread
Lactuca sativa	Lettuce	2.5.5	Substantial but local
Daucus carota spp. sativus	Carrot	4.2.4	Substantial but local
Brassica napus	Oilseed rape	2.5.3	Low but local
Brassica rapa	Turnip	2.4.3	Low but local
Raphanus sativus	Radish	3.3.3	Low but local
Cichorium intybus	Chicory	4.3.3	Low but local
Secale cereale	Rye	4.3.2	Minimal effect
Cichorium endivia	Endive	2.2.3	Minimal effect
Brassica oleracea	Cabbage	3.3.3	Minimal effect
Trifolium pratense	Red clover	5.3.1	No effect
Trifolium repens	White clover	5.3.1	No effect
Beta vulgaris	Sugar beet	1.2.1	No effect
Solanum tuberosum	Potato	5.1.0	No effect
Lycopersicon esculentum	Tomato	0.1.0	No effect
Triticum aestivum	Wheat	4.2.2	No effect
Hordeum vulgare	Barley	4.2.2	No effect
Zea mays	Maize	4.0.0	No effect

4.6.2.8 Risk categories for the 22 crops important to Switzerland



		Dp code					
Df code	Dd code	0	1	2	3	4	5
0	0	░	░	░	░	▒	▒
	1	░ tomato	░	░	░	▒	▒
	2	░ tobacco	░	░	░	▒	▒
	3	░	░	░	░	▒	▒
	4	░	░	░	░	▒	▒
	5	░	░	░	░	▒	▒
1	0	░	░	░	░	▒	▒
	1	░	░	░	▒	▒	▒
	2	░	░ beet	░	▒	▒	▓
	3	░	░	░	▒	▓	▓
	4	░	░	░	▓	▓	▓
	5	░	░	░	▓	▓	▓
2	0	░	░	░	▒	▒	▓
	1	░	░	░	▒	▒	▓
	2	░	░	▒	▒ endive	▓	▓
	3	░	░	▒	▒	▓	▓
	4	░	░	▒	▓ turnip	▓	█
	5	░	░	▒	▓	█	█ lettuce
3	0	░	░	░	▒	▒	▓
	1	░	░	░	▒	▓	▓
	2	░	░	▒	▒ cabbage	▓	█
	3	░	░	▒	▓ radish	█	▤
	4	░	░	▒	▓	█	▤
	5	░	░	▒	▓ rape	█	▤
4	0	░ mays	░	░	▒	▓	▓
	1	░	░	░ barley	▒	█	█
	2	░	░	░ wheat	▒	█ carrot	█
	3	░	░	▒ rye	▓ chicory	█	▤
	4	░	░	▒	▓	█	▤
	5	░	░	▒	▓	█	▤
5	0	░	░	░	▒	▓	█
	1	░ potato	░	▒	▓	█	▤
	2	░	░	▒	▓	█	▤
	3	░	░ clovers	▒	▓	█	▤
	4	░	░	▒	▓	█	▤ **alfalfa**
	5	░	░	▒	█	▤	▤ **grasses**

░ **No effect**
▒ **Minimal effect**
▓ **Low but local effect**
█ **Substantial but local effect**
▤ **Substantial and wide-spread effect**

The following combinations of the tree Codes are possible, each one applicable to a given crop:

4.6.2.9 Preliminary examples of risk assessment for Swiss crop plants

As an illustration, several examples are given here (a full risk assessment with these codes will be given in the publication of the final report about the above mentioned Priority Programme Project)

Alfalfa (Medicago sativa) with transgene causing rapid spread under agricultural conditions:
Categories Df 5, Dd 4, Dp 5, which means no field release of trangenic alfalfa as described above, is possible. Medium term monitoring under strict confinement conditions is necessary in order to find out about competitiveness of the transgenic variety and in order to experiment with less risky transgenes having the same favourable effect desired. No field releases to be permitted until case is solved in such a way, that transgenic alfalfa varieties has been proved to be in lower risk categories. Although we judge field trials to be premature in Europe, there have been given in the USA as many as 15 permits for insect resistance, herbicide tolerance and virus resistance (Snow et al. 1995).

Barley (Hordeum vulgare)
Categories Df 4, Dd 2, Dp 2, which means no effect with field releases of transgenic barley.
Field releases of transgenic barley possible. Certain transgenes have to be tested in medium term field experiments regarding their secondary effects on ecosystems: Insect resistance genes could well be designed in such a way, that pest insect species develop themselves too rapidly resistant populations. Field experiments should be carried out with less effective expression of the gene or with a mixture of different transgenic varieties expressing various resistances. Another strategy would be to introduce non-transgenic populations of the crop in order to produce refugial non-resistant pest insect populations. Number of permits in the USA for herbicide tolerance and virus reistance: 2 (Snow et al. 1995)

Oilseed rape *(Brassica napus)*
Categories Df 2, Dd 5, Dp 3, which means low but local effect with field releases of transgenic oilseed rape, since gene flow occurs towards a wild (feral ?) species which does not occur outside agricultural environment and control. Number of permits in the USA: 35 for herbicide tolerance, industrial enzymes produced, insect resistance, product quality (Snow et al. 1995).

These examples will be given in extenso in our final report for the SPP Project "Risk assessment of transgenic crops in Switzerland", where we will treat some 20 crops and their wild relatives in Switzerland.

These examples are restricted to transgenes which cause favourable effects regarding herbicide treatment (herbicide tolerance) and which do not cause enhanced competitiveness outside agricultural environment. Any other transgenes should be carefully studied regarding their effect on the competitiveness of the transgenic oilseed rape. Such experiments should be done in confinement.

4.6.3 How to proceed in risk assessment

There is no great hope to solve problems in risk assessment with consensus technique, since these techniques are successful in legislation on a political level. Risk assessment in field release of transgenic plants has to cope with concrete problems in a concrete environment. Here planning methodology of the second generation will be the solution.
There are several ground rules to be followed:

- Symmetry of knowledge (or ignorance): Experts and lay persons have different kinds of knowledge. If both parts learn to respect this rule, they will also start to listen to each other.
- All key role players should be invited to the discussions, they should all feel free to play their role and to speak out about their genuine interests.
- Only a step to step approach will be successful, the discursive process should be accompanied by professional moderators and also computer aid has to be considered seriously in order to keep track to the argumentative process, to document the pro's and the con's and to be able to reduce and enlarge the catalogue of critical questions and arguments.
- Most important is the circumscription of the problem: The periphery of the problematic case has to be defined precisely, this does not mean to describe the problem precisely, which would mean a major step towards problem solution already.
  In our case of problems to be solved this would mean to
  - list up all related species having possible gene flow with the transgenic cultivar
  - to define a given region (and thus a given biogeographical situation).
  - to deal with specific transgenes
  - to invite all partners affected by future field releases.
  - follow a step by step procedure, which could be structured following the scheme proposed by Rissler and Mellon (1993) (cited in Snow et al. 1995)

4.6.4 Summary of scheme to assess two environmental risks

All crops and related wild species, which belong to the two highest risk categories (substantial but local effect, substantial and widespread effect), should be treated according to the following scheme in a medium to long term monitoring with an experimental approach. Crops belonging to the highest category of risk should be treated according to a test procedure proposed by Fredshavn et al. 1993, which can be carried through greenhouses. Competition experiments in confinements will reveal data in the influence of plant size on competitiveness, on substitution rates as a measure of competitiveness and on the interaction between habitat and gene expression. But even with these preliminary greenhouse experiments it will be impossible to predict the exact ecological consequences of a release. It is, however, possible to test a transgenic plant in a confinement in critical phases of the life cycle and compare it with a range of non-transformed well-known varieties, and thus detect any principal changes in growth behavior. A set of standard growth conditions is proposed. If these experiments do not reveal any major change in the competitiveness of the transgenic crop, then the field experiment procedures according to Rissler and Melon (1993) can be started.
This approach involves a three step analysis to evaluate both crop weediness and gene flow. The step are designed to identify non-risky plants early in the analysis and to require extensive field testing only for plants that appear to pose substantial risks. The evaluation proceeds under the assumption that crops on the lower end of the spectrum of weediness potential are sufficiently unlikely to be converted to weeds by the addition of transgenes that they can be subject to simplified population replacement experiments.

The first step assesses :

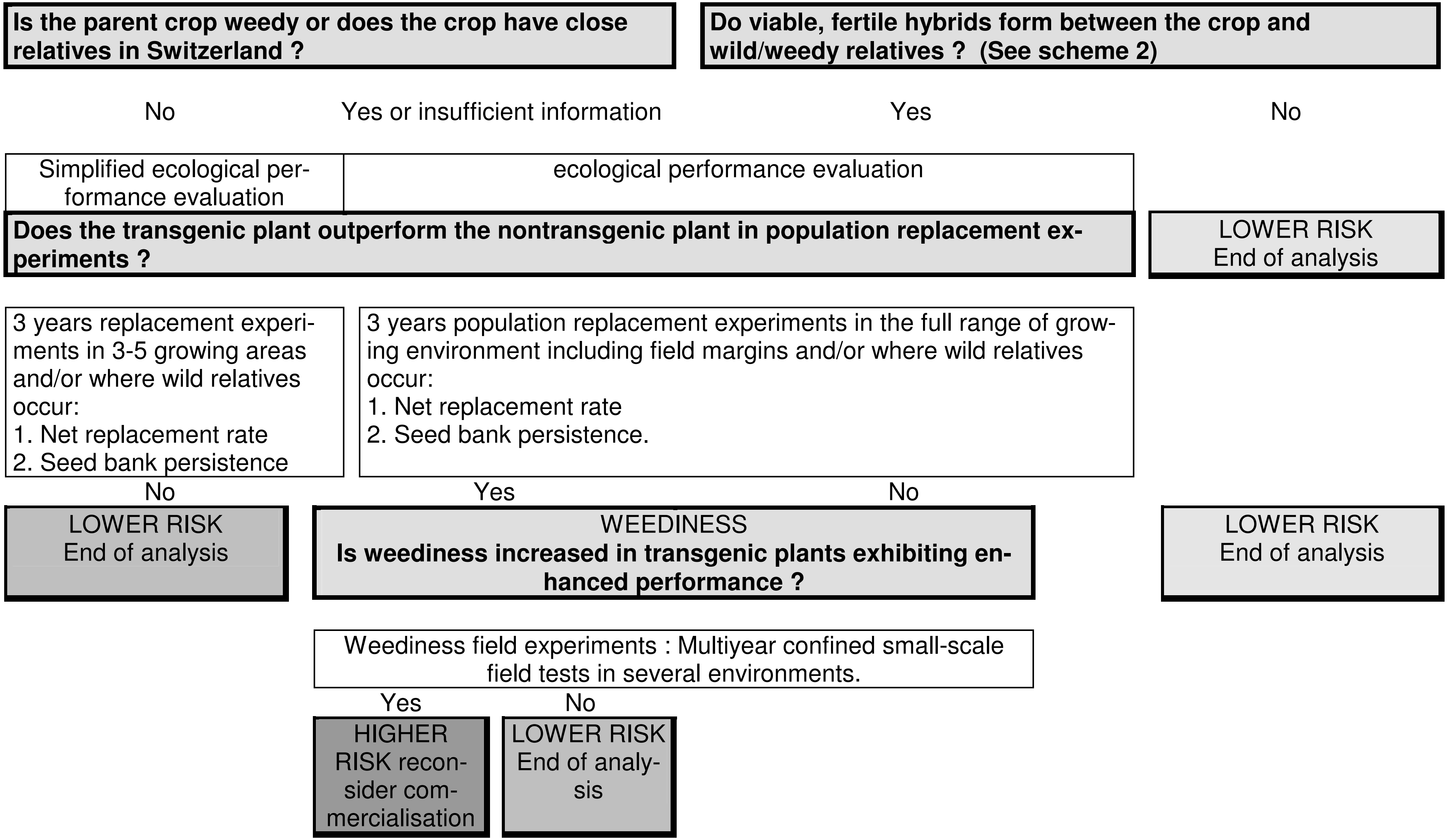
1. The potential for weediness. It separates crops into two risk categories. The lower-risk category contains crops that are not weedy and do not have close weedy relatives in Switzerland. By contrast, the higher-risk group is weedy or has close weedy relatives. The higher-risk crops are subject to a standard set of experiments, while the ones with lower-risk undergo an abbreviated procedure.
2. Experimental assessment of the potential for transgene flow determines whether transgenic hybrids will form between transgenic crops and their wild/weedy relatives. Where hybrids are not formed, the transgenic crop is deemed to pose low risk in terms of gene flow and no further tests are required. Where hybrids are formed, the analysis moves to step 2. Once gene transfer occurs, the assessment of potential adverse impacts is the same as for the transgenic crop itself.

The second step analysis relies on relatively simple experiments, which can be conducted along with efficacy tests, to evaluate the performance of transgenic crops relative to non transgenic ones.
The third step analysis allows developers an opportunity to demonstrate that transgenic crops that outperform non-transgenics in the ecological performance tests do not pose risks as weeds under conditions of commercial use.

The proposal of Rissler and Mellon (1993) are summarized in scheme 1 and 2 below

ASSESSING THE POTENTIAL FOR TRANSGENIC CROPS TO BECOME WEEDS

ASSESSING THE POTENTIAL FOR TRANSGENE FLOW TO PRODUCE WEEDS.



Is the parent crop weedy or does the crop have close relatives in Switzerland ?

Do viable, fertile hybrids form between the crop and wild/weedy relatives ? (See scheme 2)

No

Yes or insufficient information

Yes

No

Simplified ecological performance evaluation

ecological performance evaluation

Does the transgenic plant outperform the nontransgenic plant in population replacement experiments ?

LOWER RISK
End of analysis

3 years replacement experiments in 3-5 growing areas and/or where wild relatives occur:
1. Net replacement rate
2. Seed bank persistence

3 years population replacement experiments in the full range of growing environment including field margins and/or where wild relatives occur:
1. Net replacement rate
2. Seed bank persistence.

No

Yes

No

LOWER RISK
End of analysis

WEEDINESS
Is weediness increased in transgenic plants exhibiting enhanced performance ?

LOWER RISK
End of analysis

Weediness field experiments : Multiyear confined small-scale field tests in several environments.

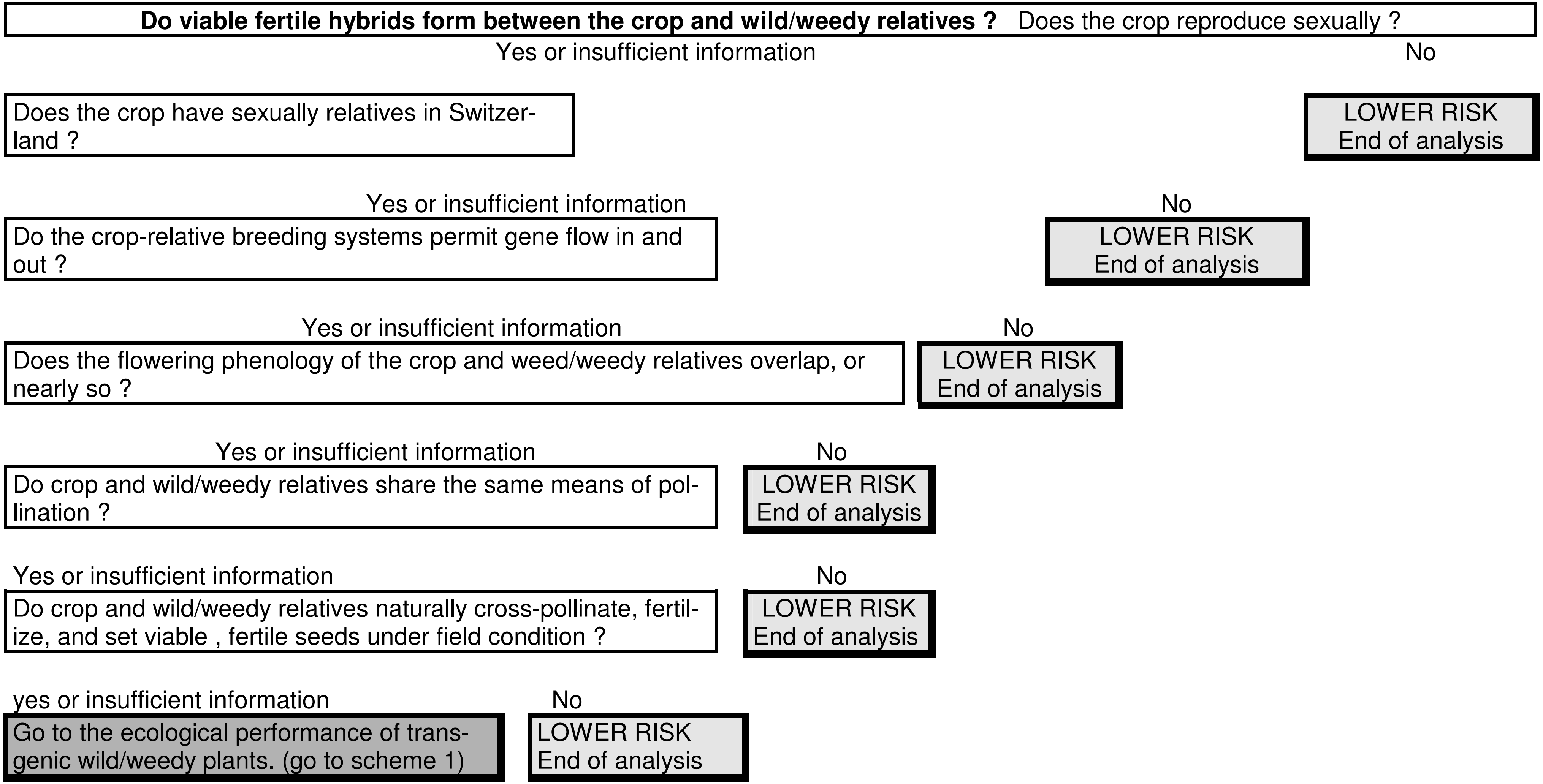
Yes

No

HIGHER RISK reconsider commercialisation

LOWER RISK
End of analysis

There are a number of ways to evaluate the replacement capacity of a genetic type in a population of plants. Option A describes one alternative. N.Ellstrand, J.Hancock, P.Kareiva, R.Linder, R.Manasse and M.L.Roush were especially helpful in developing this experimental approach given by Rissler and Mellon (1993). Here we cite it from Snow et al. (1995), appendix 3:



Do viable fertile hybrids form between the crop and wild/weedy relatives ? Does the crop reproduce sexually ?

Yes or insufficient information

No

LOWER RISK
End of analysis

Does the crop have sexually relatives in Switzerland ?

Yes or insufficient information

No

LOWER RISK
End of analysis

Do the crop-relative breeding systems permit gene flow in and out ?

Yes or insufficient information

No

LOWER RISK
End of analysis

Does the flowering phenology of the crop and weed/weedy relatives overlap, or nearly so ?

Yes or insufficient information

No

LOWER RISK
End of analysis

Do crop and wild/weedy relatives share the same means of pollination ?

Yes or insufficient information

No

LOWER RISK
End of analysis

Do crop and wild/weedy relatives naturally cross-pollinate, fertilize, and set viable , fertile seeds under field condition ?

yes or insufficient information

No

LOWER RISK
End of analysis

Go to the ecological performance of transgenic wild/weedy plants. (go to scheme 1)

4.6.5. Conclusion

The hundreds of small-scale field tests in order to evaluate the performance of genetically engineered crop varieties are up to now not designed to investigate the ecological risks of widespread commercialization (1994 International Symposium on the Biosafety Results of Field Tests of Genetically Modified Plants and Microorganisms in Monterey, CA, USA).

In order to achieve sustainability in cultivating transgenic crops, the focus should be on long term monitoring of several years in the same field where the transgenic crop was planted. To assess invasiveness, the transgenic plant's capacity to disperse and establish in adjacent and nearby habitats should be investigated.

If genetic exchange between transgenic crops and wild relatives has weediness potential, there should nearly always be evidence of this process with non transgenic crop/weed complexes. Recent evolution of weed beets in France [Boudry et al., 1993] demonstrate the novelty and effectiveness of certain fitness-related transgenes. Closer attention should be paid to possible effects on free-living wild relatives.

Rigorous studies of the sexual compatibility of crops and wild relatives are clearly needed to determine whether escaped transgenes are likely to persist in wild populations. Further research is also needed to predict how escaped transgenes are likely to affect the abundance and invasiveness of the transgenic hybrids.

Literature cited

Abbott R. 1994a. Ecological risks of transgenic crops. TREE 9, 8 August, 280-281

Abbott R. 1994b. Reply to Thacker J.R.M. Transgenic plants. TREE 9, 12 December, 486

Adams W.T., Griffin A.R. and Moran G.F. 1992. Using paternity analysis to measure effective pollen dispersal in plant populations. The American Naturalist 140,5, 762-780.

Ahl Goy P., Chasseray E. and Duesing J. 1994. Field trials of transgenic plants: an overview. Agro-Industry hi-tech March/April, 10-15.

Ahl Goy P. and Duesing J. 1995. From Plots to Plots: Genetically Modified Plants on Trial. Biotechnology 13, May, 454-458.

Ahl Goy P. and Duesing J. 1996. Assessing the Environmental Impact of Gene Transfer to Wild Relatives. Biotechnology 14, January, 39-40.

Altmann M. 1994. Schlussfolgerungen aus dem Technikfolgenabschätzungs-Verfahren (TA) zum Anbau von Kulturpflanzen mit gentechnisch erzeugter Herbizidresistenz. Gaia, 3(6):

Altmann M. and Ammann K. 1992. Gentechnologie im gesellschaftlichen Spannungsfeld: Züchtung transgener Kulturpflanzen. Gaia, 1(4): 204-213.

Ammann K. 1994. Die ökologischen Risiken der Gentechnologie und wie wir damit umgehen können. In: Ethik und Strahlenschutz, Seminar des Fachverbandes für Strahlenschutz e.V. und der Eidgenössischen Kommission für Strahlenschutz, Publikationsreihe Fortschritte im Strahlenschutz, 16-26.

Ammann K. 1995. Gentechnisch veränderte Kulturpflanzen in der Umwelt : Chancen und Probleme- Die Suche nach gangbaren Wegen. In : Gentechnik und Ernährung. Erbersdobler H.F., Hammes W. and Jany K.-D., Wissenschaftliche Verlagsgesellschaft mbH Stuttgart, 161-195.

Ammann K., Felber F., Keller [Senften] J., Jacot Y., Küpfer Ph., Rufener [Al Mazyad] P. and Savova D. 1994: Dynamic biogeography and natural hybridization of selected weedy species in Switzerland. Symposium "Gene Transfer: Are Wild Species in Danger?", Le Louverain, Switzerland, Nov.1994. Environmental Documentation, Federal Office of Environment, Forests and Landscape (FOEFL), 12: 36-39.

Anderson Ch. 1992. Researchers ask for help to save key biopesticide. News. Nature 355, 20 February, 661

Anderson E. 1949. Introgressive hybridisation. John Wiley & Sons, Inc., New York, Chapman & Hall, Limited, London.

Andow D.A. 1994. Community response to transgenic plant release: using mathematical theory to predict effects of transgenic plants. Molecular Ecology 3, 65-70.

Arnold M.L. and Hodges S.A. 1995. Are natural hybrids fit or unfit relative to their parents ? TREE vol. 10, no. 2 February, 67-71.

Ashman T.-L., Galloway L.F. and Stanton M.L. 1993. Apparent vs. effectife mating in an experimental population of Raphanus sativus. Oecologia 96, 102-107.

Austin R.B., Flavell R.B., Henson I.E. and Lowe H.J.B. 1986. Molecular biolgy and crop improvement. Cambridge University Press.

Bainton J.A. 1993. Considerations for release of herbicide resistant crops. Aspects of Applied Biology 35, Volunteer crops as weeds, 45-52.

Baker H.G. 1967. Characteristics and modes of origin of weeds. In : The genetics of colonizing species. Baker H.G. and Stebbins G.L. (eds.). Academic Press. New York and London,147-172.

Baker H.G. 1974. The evolution of weeds. Ann. Rev. of Ecology and Systematics 5: 1-24

Barrett S.C.H. 1983. Crop mimicry in weeds. Econ. Bot., 37 (3), 255-282.

Barrett J. ca. 1992. Biotechnology - Promises and Challenges for Sustainable Agriculture. OECD Workshop, Biotechnology and Practice. Manuscript.

Bartsch D. 1995. Ökologische Begleitforschung zu rizomaniaresistenten Zuckerrüben. In: Ökologie transgener Nutzpflanzen, Gentechnologie - Chancen und Risiken. Albrecht, S., Beusmann, V. (eds). Campus. Verlag Frankfurt/New York, 31: 81-98.

Bartsch D, Sukopp H. and Sukopp U. 1993. Introduction of plants with special regard to cultigens running wild. In: Transgenic organisms. Wöhrmann, K. and Tomiuk, J. (eds), Birkhäuser Verlag Basel/Switzerland, 135-151.

Bazin M.J. and Lynch J.M. 1995. Environmental Gene Release. Models, Experiments and Risk Assessment. Chapman & Hall, New York, 166p, ISBN 0-412-54630-2.

Becker Th. 1951. Siebenjährige blütenbiologische Studien an den Cruziferen Brassica napus L., Brassica rapa L., Brassica oleracea L., Raphanus L. und Sinapis L.. Teil I. Zeitschrift für Pflanzenzüchtung, 29: 222-240.

Bevan M.W., Harrison B.D. and Leaver C.J. 1995. The Production and Uses of Genetically Transformed Plants, Chapman & Hall, New York.

Blanck K.-D. 1991. Raps in der Fruchtfolge nicht überziehen! In: Rapsanbau für Könner - Bestände gezielt aufbauen und schützen. Top agrar - Das Magazin für moderne Landwirtschaft. Landwirtschaftsverlag Münster-Hiltrup, 94-98, ISBN 3-7843-1386-8.

Boudry P., Mörchen M., Samitov-Laprade P., Vernet P. and Van Dijk H. 1993. The origin and evolution of weed beets: consequences for the breeding and release of herbicide-resistant transgenic sugar beets. Theor. Appl. Genet., 87: 471-478.

Boyle-Makowski R.M.D. 1985. Pollinator activity and abiotic factors in an apple orchard. The Canadian Entomologist 117, 1509-1521.

Bryant J. 1992. Removal of selectable marker genes from transgenic plants: needless sophistication or social necessity? Tibtech 10 (8):

Crawley M.J. 1992. The comparative ecology of transgenic and conventional crops. In: 2nd international symposium on the biosafety results of field tests of genetically modified plants and microorganisms. May 11-14, 1992, Goslar, Germany, 43-52.

Crawley M.J., Halls R.S., Rees M., Kohn D. and Buxton J. 1993. Ecology of transgenic oilseed rape in natural habitats. Nature, 363(6430): 620-623. See also comments of Peter Kareiva in same Vol. p. 580. (1993).

Curtis H. 1983. Biology. New York : Worth Publishers, Inc.

Cussans G.W. 1978. The problem of volunteer crops and some possible means of their control. Proc. British Crop Protection Conference. Weeds, 3: 915-921.

Dale Ph. 1992. Spread of Engineered Genes to Wild Relatives. Plant Physiol., 100, 13-15.

Darmency H. and Renard M. 1992. Efficiency of safety procedures in experiments with transgenic oilseed rape. In: 2nd international symposium on the biosafety results of field tests of genetically modified plants and microorganisms. May 11-14, 1992, Goslar, Germany, 54-60.

Department of the Environment and Advisory Committee on Releases to the Environment 1994. Newsletter, 2: 2-5.

Dumas C. 1984. Ecologie florale et pollinisation. In : Pollinisation et production végétales. Pesson P. et Louveaux J. (eds.). INRA, Paris, 31-46

Eijlander R. 1989. Transgene biet, ja of nee. Prophyta, 43: 232-233.

Ellstrand N. 1988. Pollen as a vehicle for the escape of engineered genes ? Trends in Ecology and Evolution, 3: S30-S32.

Ellstrand N. 1992. Gene flow by pollen : implications for plant conservation genetics. Oikos, 63: 77-86.

Ellstrand N. and Marshall D. 1985. Interpopulation gene flow by pollen in wild radish, Raphanus sativus. American Naturalist, 126: 606-616.

Ellstrand N., Devlin B. and Marshall D. 1989. Gene flow by pollen into small populations : data from experimental and natural stands of wild radish. Proceedings of the National Academy of Sciences, 86: 9044-9047.

Feitelson J., J.Payne and L.Kim 1992. Bacillus thuringiensis: insects and beyond. Bio/Technology 10, 438-442.

Ferre J., M.D.Real, J.Van Rie, S.Jansens and M.Peferoen 1991. Resistance to the Bacillus thuringiensis bioinsecticide in a field population of Plutella xylostella is due to a change in a midgut membrane receptor. Proc. Nat. Acad. Sci. USA 88, 5119-5123.

Fitter A., Perrins J. and Williamson M. 1990. Weed probability challenged. Bio/Technology 8: 473.

Fredshavn J.R., Poulsen G.B., Madsen K.H. and Jensen S.M. 1992. Safety assessment of deliberate release of transgenic sugar beet II. In: BRIDGE, first sectoral meeting on biosafety. Wageningen, December 6-9, 1992. Commission of the Eruropean Communities, Brussels, Belgium and Wageningen Agricultural University, Wageningen, The Netherlands, 41.

Fredshavn J. and Poulsen G.S. 1993. Growth behaviour and competitive ability of transgenic crops. Agric. Ecosystems Environ.

Fredshavn J.R. and Poulsen G.S. 1995. Competitiveness of transgenic oilseed rape. Transgenic Research 4, 142-148.

Friedt W. 1995. Oral presentation 'Erwünschte und unerwünschte Inhaltsstoffe - eine Herausforderung für den Pflanzenbau' at the annual meeting 1995 of the Swiss Society of Agronomy in Bern, Switzerland.

Frietema De Vries F.T. 1996. Cultivated plants and the wild flora. Effect analysis by dispersal codes. Thesis. Rijksherbarium, Hortus Botanicus, Leiden, 100p.

Frietema De Vries F.T., Van der Meidjen R. And Bradenburg W.A. 1994. Botanical files on lettuce (*Lactuca sativa* L.) for gene flow between wild and cultivated lettuce (*Lactuca sativa* L. Including *L. Serriola* L., Compositae) and the generalized implications for risk assessment on Genetically Modified Plants. Gorteria, suppl. 2, 44p.

Gabriel W. 1993. Technologically modified genes in natural populations: some skeptical remarks on risk assessment from the view of population genetics. Transgenic Organisms. K. Wöhrmann and J. Tomiuk (eds.). Birkhäuser, Basel 1993, 109-116.

Giddings G. 1995. Genetically engineered crops : What risks ? (first draft).

Gold T.J. et al. 1991. Agrobacterium rhizogenes mediated transformation of the forage legumes Medicago sativa and Onobrychis viciifolia. J.Exp. Botany, 42, 1147-1157.

Gould F. 1988a. Evolutionary Biology and Genetically Engineered Crops. Consideration of evolutionary biology can aid in crop design. BioScience 38,1, January, 26-32.

Gould F. 1988b. Genetic engineering, integrated pest management and the evolution of pests, TREE 3,4, April 15-18.

Gould F., Martinez-Ramirez A., Anderson A., Ferre J., Silva F. and Moar W.1992. Broad Spectrum resistance to Bacillus thuringiensis toxins in Heliothis virescens

Grallien E., Marilleau R., Pham-Delègue M.H. and Picard-Nizou A.L. 1995. Impact of pest insect resistant oilseed rape on honeybees. In :Rapeseed today and tomorow, 9th International rapeseed congress, Cambridge, UK, 4-7 july 1995, 3: 784-786.

Graner A. and Wenzel G. 1993. Chancen und Risiken der Grünen Gentechnik. Pflanzenschutz-Praxis, 4: 20-22.

Grant V. 1981. Plant speciation. Second edition. New York, Columbia University Press.

Gray 1986

Green Industry Biotechnology Platform 1995. Extracts from GIBiP Database on Field Trials 1986 - 1994, G95/129

Gregorius H.-R. and Steiner W. 1993. Gene transfer in plants as a potential agent of introgression, In: Transgenic Organisms. K. Wöhrmann and J. Tomiuk (eds.), Birkhäuser, Basel 1993, 109-116.

Gressel J. 1993. Management of herbicide -resistant weeds in crop production. In : International Crop Science I., Crop Science Society of America , 677S, Madison, USA.

Gressel J. Indiscriminate use of selectable markers - sowing wild oats ? TIBTECH, in press.

Gressel J., Regev Y., Malkin S. and Kleifeld Y. 1983. Characterization of an s-triazine-resistant biotype of Brachypodium distachyon. Weed Sci. 32: 450-456.

Gressel J. and Kleifeld Y. 1994. Can wild species become problem weeds because of herbicide resistance? Brachypodium distachyon: a case study. Crop protection, 13 (8): 563-566.

Hadley H.H. and Openshaw S.J. 1980. Interspecific and intergeneric hybridization. In . Hybridization of crop plants. Fehr. W.R. and Hadley H.H. (eds.). American Society of Agronomy-Crop Science Society of America, Madison, 133-159.

Halliun d' K., Sottermann J. and Greef de W. 1990. Engineering of herbicide resistant alfalfa and evaluation under field conditions. Crop Science, 30: 866-871.

Halluin D' K., Bossut M., Bonne E., Mazur B., Leemans J. and Botterman J. 1992. Transformation of sugarbeet (*Beta vulgaris* L.) and evaluation of herbicide resistance in transgenic plants. Bio/Technology 10: 309-314.

Harding K. and Harris P.S. 1994. Risk assessment of the release of genetically modified plants : a review. Edited by Ministry of Agriculture, Fisheries and Food, London, 54pp.

Harlan J.R. 1965. The possible role of weed races in the evolution of cultivated plants. Euphytica, 14: 173-176.

Harlan J.R., De Weet J.M.J. and Price E.G. 1973. Comparative evolution of cereals. Evolution, 27, 311-325.

Hartmann E., Schuldes H., Kübler R. and Konold W. 1994. Neophyten - Biologie, Verbreitung, Kontrolle ausgewählter Arten. Ecomed. Landsberg, Germany, 302p, ISBN 3-609-65450-3.

Heywood V. and Zohary 1995. A Catalogue of the Wild Relatives of Cultivated Plants Native to Europe. Flora Mediterranea 5: 375-415.

Hill K.K. et al. 1991. The development of virus-resistant alfalfa, Medicago sativa L. Bio/Technology, 9: 373-377.

Holm L.G. et al. 1977. The world’s worst weeds. Distribution and biology. University Press of Hawaii, Honolulu.

Holzner W. 1982. Concepts, categories and characteristics of weeds. In: Holzner, W. and Numata, N. (eds), Biology and ecology of weeds, Dr. W. Junk Publishers, The Hague, 3-20.

Hoyle R. 1993. Herbicide-resistant crops are no conspiracy. BIO/TECHNOLOGY ,11: 783-78.

Jacot Y. and Jacot P. 1994. Application du génie génétique à l’agriculture : Evaluation des dangers potentiels pour la flore suisse. Cahier de l’Environnement, Organismes, N° 235, 77p.

Jörgensen R.B. 1993. Genetic pollution in the genus *Brassica.* A case story of the gene flow between oilseed rape (*B. napus*) and wild *B. campestris. [B. rapa subsp. campestris]* Council of Europe, workshop on conservation of the wild relatives of European cultivated plants: developing intergrated strategies. Neuchâtel University (Switzerland), working document.

Jörgensen R.B. and Andersen B. 1994. Spontaneous hybridization between oilseed rape (*Brassica napus*) and weedy *B. campestris [B. rapa subsp. campestris]* (*Brassicaceae*): a risk of growing genetically modified oilseed rape. American Journal of Botany 81(12): 1620-1626.

Joergensen R.B., Andersen B., Landbo L., Michelsen T. 1995. Spontaneous hybridization between oil seed rape (*B.napus*) and weedy relatives. Actae Horticulturae, in press.

Keeler K.and Turner Ch. 1991. Management of Transgenic Plants in the Environment. In: Risk Assessment in Genetic Engineering, Levin M. and H. Strauss, eds.). Mc Graw Hill, 189-218.

Kirkpatrick K.J. and Wilson H.D. 1988. Interspecific geneflow in Cucurbita : C. texana vs. C. pepo. Amer. J. Bot., 75: 519-527.

Kjellsson G. and Simonsen V. 1994. Methods for risk assessment of transgenic plants. I. Competition, establishment and ecosystem effects. Birkhäuser, Basel.

Lambelet-Haueter C., 1990. Mauvaises herbes et flore anthropogène. I. Définitions, concepts et caractéristiques écologiques. Saussurea 21: 47-73.

Lambelet-Haueter C. 1991. Mauvaises herbes et flore anthropogène. II. Classifications et catégories. Saussurea 22: 49-81.

Langevin S.A., Clay K. and Grace J.B. 1990. The incidence and effects of hybridisation between cultivated rice and its related weed red rice (Oryza sativa L.). Evolution, 44: 1000-1108.

Larkin P.J. et al. 1990. Direct gene transfer to protoplasts. Australian J. of Plant Physiology, 17: 291-302.

Leisinger K. 1991. Gentechnik für die Dritte Welt ? Birkhäuser , Basel.

Levin D. 1978. The origin of isolating mechanisms in flowering plants. In : Evolutionary Biology. Hecht M., Steere W. and Wallace B. (eds.). Plenum Press, New York, 185-317.

Levin D.A. and Kerster H.W. 1974. Gene flow in seed plants. Evol. Biol., 7: 139-220.

Lupi C. 1995. Genetic engineering for plant protection - Methods, state of the art and applications. Technology assessment of pest resistant transgenic plants, report 1. BATS, 60p.

Madsen K.H 1994. Weed management and impact on ecology of growing glyphosate tolerant sugarbeets (*Beta vulgaris* L.). Ph.D.-Thesis. The Royal Veterinary and Agricultural University, Weed Science, Copenhagen, Denmark and MARIBO Seed, Holeby, Denmark, 61p.

Mallet J. and Porter P. 1992. Preventing insect adaptation to insect-resitant crops: are seed mixtures or refugia the best strategy? Proc. R. Soc. 165-169, London.

Mandel M.A. et al. 1992. Cell 71, 133-143.

Mangelsdorf P.C. 1961. Introgression in Maize. Euphytica, 10: 157-168.

McGaughey W.H. 1985. Insect resistance to the biological insecticide Bacillus thuringiensis. Science 229, 193-194.

McGaughey W.H., William M.E. and Wilson E. 1992. Managing Insect Resistance to Bacillus thuringiensis Toxins. Science 258, 1451-1455.

Meer Van der P.J. 1993. Proceedings (working dokument) Pan-European conference on the potential long-term ecological impacts of the release of genetically modified organisms. Council of Europe, Strasbourg 1993.

Mikkelsen T.R., Landbo L. and Joergensen R.B. 1995. Interspecific flow of transgenes in field experiments. In : Workshop on *Brassica napus*, Book of abstracts, 20-23 march 1995, Helsingor, Denmark, L8.

Mikkelsen T.R., Andersen B. and Jörgensen R.B. 1996. The risk of crop transgene spread. Nature 380, 7 March, 31.

Mizukami Y. and ma H. 1992. Cell 71, 119-131.

Morris W.F., Kareiva P.M. and Raymer P.L. 1995. Ecol Appl. (in press)

Newmann S.M. 1990. Pollen transfer from transgenic plants : Concepts for risks assessment and data requirements. AAAS/EPA Environmental Science and Engineering Fellow, 1-40.

NRC Report on Field Testing 1989. National Research Concil of the National Academy of Sciences. Committee on scientific evaluation of the introduction of genetically modified micro-organisms and plants into the environment. *Field testing genetically modified organisms: Framework for decisions*. National Academy Press, Washington, D.C., 170 p, ISBN 0-309-04076-0.

OECD 1993a. Field releases of transgenic plants, 1986-1992 an analysis. OECD, Paris, 39p. ISBN 92-64-14046-8.

OECD 1993b. Safety considerations for biotechnology: Scale-up of crop plants. OECD, Paris, 41p. ISBN 92-64-14044-1.

Oka H.-I. and Morishima H., 1982. Ecological genetics and the evolution of weeds. In: Holzner, W. and Numata, N. (eds), Biology and ecology of weeds. Dr. W. Junk Publishers, The Hague, 73-89.

Omirulleh S. et al. 1993. Plant Mol. Biol., 21, 415-428.

Paul E.M., Lewis G.b. and dunwell J.M. 1991. The pollination of genetically modified plants. Acta Horticulturae, 288, 425-429.

Pham-Délègue M.H., Picard-Nizou A.L., Arnorld G. Grison R., Toppan A., Olsen L. and Masson C. 1992. Impact of genetically modified rapeseed on insect pollinators (honeybees). In : Proceedings of the 2nd International

Symposium on „the biosafety results of field tests of genetically modified plants and micro-organisms", May 11-14, 1992, Goslar, Germany, Casper R. and Landsmann J. (eds.).

Pickersgill B. 1981. Biosystematics of crop-weed complexes. Kulturpflanze, XXIX: 377-388.

Poulsen G.S. and Fredshavn J. 1995. Assessing competitive ability of transgenic oilseed rape. Workshop on *Brassica napus*, Helsingör, 20-23 march 1995, Denmark, L13.

Raamsdonk van L.W.D. 1993. Wild and cultivated plants : the paralellism between evolution and domestication. Evolutionary Trends in Plants, 7(2): 73-84.

Rauber R., 1977. Evolution von Unkräutern. Ergebnisse der 1. Deutschen Arbeitsbesprechung über Fragen der Unkrautbiologie und -bekämpfung, 1977, Stuttgart, Hohenheim. Zeitschrift für Pflanzenkrankheiten und Pflanzenschutz, Sonderheft 8: 37-55.

Raybould A. and Gray A. 1993. Genetically modified crops and hybridization with wild relatives: A UK perspective. Journal of Applied Ecology 30, 199-219

Raybould A. and Gray A. 1994. Will hybrids of genetically modified crops invade natural communities ? TREE , 9(3): 85-88.

Regal P. 1992. Scientific principles for ecologically based risk assessment of transgenic plants. Draft prepared for the symposium Potential Ecological and non-target effects of transgenic plant gene products on agriculture, silviculture and natural ecosystems. November 30-December 2, 1992, College Park, Md.

Regal P.J. 1994. Scientific principles for ecologically based risk assessment of transgenic organisms Molecular Ecology (1994) 3, 5-13.

Rhodes C.A., Pierce D.A., Mettler I.J., Mascarenhas D. and Detmer J.J. 1988. Science, 240, 204-207.

Rich T.C.G. 1991. Crucifers of Great Britain and Ireland. B.S.B.I. Handbook No. 6.. Botanical Society of the British Isles. London, ISBN 0-901-158-208.

Rissler J. and M.Mellon 1993. Perils amidst the promise: ecological risks of transgenic crops in a global market. Union of Concerned Scientists, Cambridge, M.A.

Rösler L. 1969. Zur Bestimmung und Beurteilung fon Fatuoiden und Bastardformen zwischen Saat- und Flughafer. Saat- und Pflanzgut 10: 731-735.

Salm van der, T., D.Bosch, G.Honee, L.Feng, E.Munterman, P.Bakker, W.Steikema and B.Visser 1994. Insect resistance of transgenic plants that express modified Bacillus thuringiensis cryIA(b) and dryIC genes: a resistance management strategy. Plant Molecular Biology 26, 51-59.

Schlink S. 1994. Ökologie der Keimung und Dormanz von Körnerraps (*Brassica napus* L.) und ihre Bedeutung für eine Überdauerung der Samen im Boden. Dissertationes Botanicae Band 222. Cramer, Berlin, Stuttgart, 194p, ISBN 3-443-64134-2.

Schlüter K. und I. Potrykus 1995. Horizontaler Gentransfer von transgenen Pflanzen zu Mikroorganismen (Bakterien und Pilzen) und seine ökologische Relevanz. Gutachten BATS, Fachstelle für Biosicherheit und Abschätzung von Technikfolgen des Schwerpunktprogrammes Biotechnologie, 1-39.

Scholz H. 1983. Die Unkraut-Hirse (*Panicum miliaceum* subsp. *ruderale*) neue Tatsachen und Befunde. Plant Syst. Evol., 143, 233-244.

Scholz H. 1991. Die Systematik der *Avena sterilis* und *A. fatua* (Gramineae). Eine kritische Studie. Willdenowia, 20, 103-112.

Scholz H. 1993. Kommentar zur Studie: „Ökologische Langzeit-Effekte der Verwilderung von Kulturpflanzen". Gutachten erstellt im Auftrag der Abteilung „Normbildung und Umwelt", Forschungsschwerpunkt Technik-Arbeit-Umwelt, des Wissenschaftszentrums Berlin für Sozialforschung (WZB), Berlin.

Schönberger H. and Vries De G. 1991. So bauen Sie den Rapsbestand gezielt auf. In: Rapsanbau für Könner - Bestände gezielt aufbauen und schützen. Top agrar - Das Magazin für moderne Landwirtschaft. Landwirtschaftsverlag Münster-Hiltrup, 20-26, ISBN 3-7843-1386-8.

Schroeder H.E. et al. 1991. Expression of a chiken ovalbumin gene in three lucerne cultivars. Australian Journal of Plant Physiology, 18: 495-505.

Schwaintz F. 1971. Die Entstehung der Kulturpflanzen als Modell für die Evolution der gesamten Pflanzenwelt. In: Die Evolution der Organismen, II/2. Heberer, G. (ed.). Stuttgart.

Simmonds N.W. 1976. Evolution of crop plants. Longmann, London and New York.

Simmonds N.W. 1979. Principles of crop improvment. Longman, London and New York.

Simmonds N.W. 1988. Principes d'amélioration génétique des végétaux. Les Presses Universitaires de Laval, Québec, ISBN 2-7637-7117-3

Small E. 1984. Hybridization in the domesticated weed-wild complex. In : Plant Biosystematics. Ed. Grant W.F., Academic Press, Toronto, 195-210.

Snow A.A. and P.Moran-Palma 1995. Ecological risks of cultivating transgenic plants. Article submitted to the US Environmental Agency. Dept. of Plant Biology Ohio State University, September 1, final version, 1-

Somers D.A. et al. 1992. Transgenic plants in cultivated oat (Avena sativa L.). Abstracts of the first international Crop Science Congress, Iowa State University Ames, U.S.A., 61.

Sukopp U. and Sukopp H. 1993. Das Modell der Einführung und Einbürgerung nicht einheimischer Arten - Ein Beitrag zur Diskussion über die Freisetzung gentechnisch veränderter Arten. Gaia 2(5): 267-288.

Sukopp U. and Sukopp H. 1994. Ökologische Lang-Zeiteffekte der Verwilderung von Kulturpflanzen. Verfahren zur Technikfolgenabschätzung des Anbaus von Kulturpflanzen mit gentechnisch erzeugter Herbizidresistenz. Heft 4. TU Berlin, 144p.

Tabashnik B.E. 1994. Evolution of resistance to Bacillus thuringiensis. Annual Reviev of Entomology 39, 47-79

Tabashnik B.E., Finson N. and Johnson M.W. 1991. Managing resistance to Bacillus thuringiensis: lessons from the diamond back moth (Lepidoptera: Plutellidae). J. Econ. Entomol. 84, 49-55

Tabashnik B.E., Schwartz J.M., Finson N. and Johnson M.W. 1992. J. Econ. Entomol. 85, 1046-1055

Thacker J.R.M. 1994. Transgenic plants. TREE 9, 12 December, 486

Theisen H. 1991. Bio- und Gentechnologie - Eine politische Herausforderung., Kohlhammer, Stuttgart, S193.

Thellung A. 1912. La flore adventice de Montpellier. Mem. Soc. Nation. Sci. Nat. Math. 38: 57-728.

Tiedje J.M., Colwell R.K., Grossmann Y.L., Hodson R.E., Lenski R.E., Mack R.N. and Regal Ph.J. 1989. The planned introduction of genetically engineered organisms: Ecological considerations and recommendations. Ecology 70,2, 298-315.

Timmons A.M., Charters Y.M., Crawford J.W., Burn D., Scott S.E., Dubbels S.J., Wilson N.J., Robertson A., O'Brien E.T., Squire G.R., Wilkinson M.J. 1996. Risks from transgenic crops. Nature 380, 11 April, p.487

Tomiuk J. and Loeschcke V. 1993. Conditions for establishment and persistence of populations of transgenic organisms. In: Transgenic organisms, Wöhrmann K. and Tomiuk J. eds., p. 117-133.

Toriyama K. et al. 1991. Theor. Appl. Genet., 81, 769-776.

Tynan J.L., Williams M.K. and Connor A.J. 1989. J. Genet. Breed., 44, 303-306.

Umbeck P.F. et al. 1991. J. Econ. Entomol., 84, 1943-1950.

Vries de F.T., van der Meijden R. and Brandenburg W.A. 1992. Botanical files - A study of the real chances for spontaneous gene flow from cultivated plants to the wild flora of the Netherlands. Gorteria suppl.1, 100p.

Vries de I.M. 1990. Crossing experiments of lettuce cultivars and species (Lactuca sect. Lactuca, Compositae). Plant. Syst. Evol., 171, 232-233.

Weber B. 1995. Überlegungen zur Aussagekraft von Risikoforschung zur Freisetzung transgener Pflanzen. In: Albrecht, S., Beusmann, V. (eds), Ökologie transgener Nutzpflanzen, Gentechnologie - Chancen und Risiken. Campus.Verlag Frankfurt/New York., 31: 111-126.

Welten M. und Sutter R. 1972. Verbreitungsatlas der Farn- und Blütenpflanzen der Schweiz. Vol. 1 & 2, Birkhäuser, Basel

Williams I.H., Corbett S.A. and Osborne J.L. 1991. Beekeeping, wild bees and pollination in the European community. Bee World, 72: 170-180.

Williams S.L., S.D.F., S.Dincher, N.Carozzi, H.Kessman, E.Ward and J.Ryals 1992. Chemical regulation of Bacillus thuringiensis alpha-endotoxin expression in transgenic plants. Bio/technollogy 10, 540-543.

Williamson M.H. 1988. Potential effects of recombinant DNA organisms on ecosystems and their components. TREE, 3: 32-35.

Williamson M.H. 1993. Invaders, weeds and the risk from genetically manipulated organisms. Experientia, 49: 219-224.

Williamson M., Perrins J. and Fitter A. 1990. Releasing genetically engineered plants: Present proposals and possible hazards. Trends in Ecology and Evolution 5: 417-419

Wrubel R.P., Krimsky S. and Wetzler R.E. 1992. Field Testing Transgenic Plants. An analysis of the US Department of Agriculture's environmental assessments. BioScience 42(4): 280-288.

Young S. 1989. Wayward genes play the field. New Scientist, September 1989, 49-53.

Zohary D. 1992. Reproductive biology - introduction. Council of Europe, first workshop on conservation of the wild relatives of European cultivated plants: developing intergrated strategies. Ría Formosa Nature Reserve, FARO (Portugal).

Zoldan J. 1993. Ackerunkräuter - ein Spiegel der landwirtschaftlichen Entwicklung. Spektrum der Wissenschaft, Juli 1993, 92-108